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Role of exercise-induced hepatokines in metabolic disorders.

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Exercise, Hepatokines and Metabolic Disorders

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21 **Abstract**

22 The health-promoting effects of physical activity to prevent and treat metabolic disorders are
23 numerous. However, the underlying molecular mechanisms are not yet completely
24 deciphered. In recent years, studies have referred to the liver as an endocrine organ, since it
25 releases specific proteins called hepatokines. Some of these hepatokines are involved in
26 whole-body metabolic homeostasis and are theorised to participate in the development of
27 metabolic disease. In this regard, the present review describes the role of FGF21, Fetuin-A,
28 Angiopoietin-like protein 4 and Follistatin in metabolic disease and their production in
29 response to acute exercise. Also, we discuss the potential role of hepatokines in mediating the
30 beneficial effects of regular exercise and the future challenges to the discovery of new
31 exercise-induced hepatokines.

32

33 **Introduction**

34 The prevalence of metabolic disorders is increasing worldwide and appears as a major public
35 health concern. Management of these pathologies is complicated as practitioners are facing a
36 wide range of new co-morbidities. This is exemplified by the increase of non-alcoholic fatty
37 liver diseases (NAFLD), a spectrum of chronic liver conditions including non-alcoholic fatty
38 liver (NAFL) and non-alcoholic steatohepatitis (NASH) (22). NAFL is defined as a presence
39 of $\geq 5\%$ hepatic steatosis without any sign of hepatocyte injury while NASH is present with
40 $\geq 5\%$ hepatic steatosis with inflammation and hepatocyte injury (ballooning) with or without
41 fibrosis (22). For example, it has been underlined that 75 to 100 million people in the United
42 States are suffering from NAFLD (131) and it is the most common liver abnormality in
43 children aged 2 to 19 years (166). While it is well accepted that genetic factors play a
44 significant role, poor quality of life in terms of physical activity and nutrition are also major
45 risk factors in the aetiology of these pathologies (25, 117). In most cases, changing these
46 unhealthy habits is sufficient to significantly improve the physiological profile of patients
47 with obesity, type 2 diabetes (T2D) or NAFLD (2, 95, 161). Also, emerging hypothesis
48 associate NAFLD and the pathogenesis of extrahepatic diseases such as T2D or
49 cardiovascular diseases (CVD). Indeed, elegant reviews report that NAFLD represents an
50 independent risk factor for the incidence of T2D or CVD (1, 113, 151). Moreover, it appears
51 that ectopic fat in the liver represents a stronger risk factor of different stages of prediabetes,
52 insulin resistance or CVD than total or visceral fat mass (37, 74, 153). While hepatic
53 inflammation represents a good candidate (3), the underlying mechanisms linking NAFLD
54 and extrahepatic diseases remain to be deciphered.

55

56 The beneficial effects of exercise at the whole-body level are numerous, with particular
57 reference, here, to the adaptive responses occurring in many organs conferring protection

58 against metabolic diseases, such as obesity and T2D (16). In order to optimise the prescription
59 of physical activity, investigators seek to better understand the underlying mechanisms
60 involved in the beneficial effects of exercise. Recent studies suggest that myokines, released
61 by the skeletal muscle at rest and/or during exercise, might be partially involved (35, 123,
62 165). Exercise-induced myokines can act locally to regulate skeletal muscle energy
63 metabolism by improving insulin sensitivity, mitochondrial function or inflammation.
64 Myokines also participate in the cross talk during and after exercise between skeletal muscles
65 and other organs (ie: adipose tissues, liver, pancreas). Furthermore, studies show that the
66 liver can also release proteins, referred to here as hepatokines, which may alter whole-body
67 homeostasis at rest and during exercise. Hepatokines can be either beneficial or deleterious in
68 the context of metabolic disease by regulating signalling pathways involved in energy
69 metabolism (109, 149). Interestingly, recent evidence suggests that exercise can modulate the
70 expression of some hepatokines, suggesting that the liver might also participate in tissue
71 cross-talk during physical activity (57, 60, 68).

72

73 This review aims to summarise the current literature on hepatokines and their regulation by
74 acute and chronic exercise in the context of metabolic disorders such as obesity, insulin-
75 resistance, T2D, NAFL and NASH.

76

77 **Hepatokines and metabolic diseases**

78 Liver is a major metabolic organ. It serves as a site of storage and supply of nutrients to
79 ensure metabolic homeostasis. Also, evidence supports that hepatocytes can produce and
80 secrete proteins named as hepatokines (109, 149). Early studies in the area reported that a
81 liver-derived protein, alpha 2-HS Glycoprotein, also known as fetuin-A, can inhibit insulin
82 tyrosine kinase activation and might play a role in the pathogenesis of metabolic disorders (9,

145). However, it is only recently that the progression of NAFLD worldwide (131) has generated great interest in hepatokines. Recent study from Xiong and collaborators compared the liver transcriptome and proteome of control and mice with diet-induced NASH (170). RNA-sequence and proteomic analyses revealed that a total of 156 targets were altered at both mRNA and protein levels. Moreover, the authors reported a profound reprogramming of the hepatic secretome and membrane receptor gene expression during diet-induced NASH. Thus, these results suggest that hepatokine production could remodel metabolic homeostasis. This is exemplified by a number of studies revealing that hepatokines play a pivotal role in metabolism and contribute to the development of obesity, insulin-resistance, T2D, NAFL and NASH (109, 149). So far, about twenty hepatokines have been described to be involved in the regulation of energy and nutrient metabolism by acting directly on the liver or on distal target tissues (Table 1). These proteins regulate glucose and lipid metabolism in the liver, but also in the skeletal muscle or the adipose tissue. Moreover, hepatokines participate in inflammation, beta cell function or mitochondrial function and could participate in the development of CVD. For example, the hepatokine selenoprotein P has been reported to be increased in patients with NAFLD or visceral obesity (27) and to contribute to the development of insulin resistance (110). Selenoprotein P also inhibits vascular endothelial growth factor-stimulated cell proliferation, tubule formation, and migration in human umbilical vein endothelial cells (69). Thus, hepatokines can participate in inter-tissue crosstalk and play an influential role in hepatic and extra hepatic diseases.

Hepatokines and exercise

The beneficial effects of exercise in the context of metabolic disorders are numerous. A recent meta-analysis revealed that exercise, independently of weight loss, improves hepatic steatosis (154). Researches are hence focusing on understanding the molecular mechanism mediating

the health promoting effect of regular exercise. One plausible explanation resides in exercise secreted factors. Firstly, due to its role in locomotion, research has focused on skeletal muscle. The most well-characterized myokine is Interleukin-6 (IL-6) (118, 148). Initial work reported that IL-6 is released and secreted by the contracting skeletal muscle during exercise and stimulates hepatic glucose production to ensure the energy demands of the contracting muscle are adequately met (39). Thus, muscle-derived IL-6 works as an energy sensor to increase release of energy substrates from liver and adipose tissues (124). Secondly, due to its central role in obesity-associated disorders, adipose tissue and adipokines have been investigated. While the effects of a single bout of exercise are modest, exercise training can remodel adipokine expression and secretion. In patients with type 2 diabetes, a recent meta-analysis showed that an aerobic exercise program was associated with a significant change in leptin but did not alter adiponectin levels (63). Finally, studies reported that exercise can trigger the secretion of liver-derived proteins in response to exercise. Using hepatic arterial-to-venous difference, it has been shown that a 1-h single bout of cycling increases HSP72 release from the liver (41). Also, transcriptomic analyses in the liver revealed that exercise induces changes in the mRNA of secreted proteins suggesting that exercise can impact liver secretome (64). It is now clear that a single session of exercise is accompanied by the production of liver-secreted proteins. Hepatokines can also mediate the beneficial effects of chronic exercise or, at least, represent biomarkers of training-induced metabolic improvements (table 1). Interestingly, it has also been reported that selenoprotein-P deficiency increases responsiveness to exercise in mice through upregulation of reactive oxygen species and AMP-activated protein kinase in muscle (111), suggesting that liver-secreted proteins can influence exercise capacity.

Here, we review the current literature on exercise-induced hepatokines implicated in the regulation of metabolism and metabolic diseases. Hepatokines with i) proven release from the

liver using arterial-to-venous difference over the splanchnic bed and increased hepatic mRNA expression (FGF21, Follistatin ANGPTL4) and/or ii) a clear role in the beneficial adaptation to chronic exercise (Fetuin-A) were specifically studied in this review.

Fibroblast growth factor 21 (FGF21)

FGF21 is a 24kDa protein that signals through a cell-surface receptor complex composed of a classic FGF receptor, FGFR1c, and the FGF coreceptor, β -klotho (87, 115). It appears that FGF21 is highly expressed in the liver in both rodent and human (115, 126). Also, while a broad range of tissues are expressing FGF21 (45), it should be specified that, under physiological conditions, FGF21 gene expression is increased in the liver and to a lesser extent the brain (156) and the pancreas (79).

A/ FGF21 in metabolic diseases

Since circulating FGF-21 concentrations increase with obesity (14, 179), T2D (24, 103) and NAFLD (91), FGF21 levels have been reported as a marker of metabolic disorders (122). Also, it is important to notice that, independently of BMI, hepatic triglyceride content is the strongest determinant of hepatic FGF21 production and circulating FGF21 (91, 173). Mechanistically, it appears that high fructose consumption leads to an increase of FGF21 in mice and humans through the activation of ChREBP in the liver (42). Lessons from transgenic mice have contributed considerably to our understanding of the role of FGF21 in energy metabolism regulation. Whole body FGF21 KO mice present with an impairment of glucose metabolism and an excessively abnormal body weight (11). Moreover, in diet-induced obese (DIO) mice, insulin and glucose tolerance is more impaired when mice are conditionally lacking FGF21 in the liver compared with their age- and sex-matched control littermates (101). Also, FGF21 KO mice exhibit severe hepatic insulin resistance when fed with a

ketogenic diet compared with WT controls, when assessed by the gold-standard technique, the hyperinsulinemic–euglycemic clamp. This was associated with an increase in hepatic diacylglycerol content, leading to protein kinase C ϵ activation, a well-known kinase involved in insulin signalling impairments (19, 136). Moreover, FGF21 KO mice exhibited increased hepatic steatosis and VLDLR protein content through the activation of the eIF2a-ATF4 pathway (178). Conversely, some studies have investigated the potential role for FGF21 as a therapeutic target to prevent and treat metabolic disorders. A first study revealed that 3 to 7 days of subcutaneous administration of FGF-21 to diabetic rodents led to a significant lowering of circulating glucose and triglycerides, as well as a reduction in fasted insulin levels and improved glucose clearance during an oral glucose tolerance test (80). Moreover, treatment for 12 weeks with escalating doses of FGF21 decreased body weight, improved glucose tolerance and reduced concentrations of plasma triglycerides in high fat-fed, obese monkeys (4). Amongst the effects of FGF21 upon the liver, continuous, two week infusion of FGF21 with a miniosmotic pump to diabetic rodents led to a significant decrease in hepatosteatosis (29). It appears that FGF21 treatment abolished *de novo* lipogenesis through the reduction of SREBP-1 and fatty acid synthase in DIO mice (172). Also, *in vivo* hyperinsulinemic-euglycemic clamps in obese, leptin deficient (ob/ob) mice, revealed that 8 days of FGF21 injections improved hepatic insulin sensitivity and decreased hepatic glucose output (13). In a mice model of NASH, Lee and colleagues reported that 3 weeks of injections with the FGF21 analog LY2405319 prevented oxidative stress in the liver, a key component in the development of insulin resistance (30, 82, 89). Finally, clinical trials in patients with T2D revealed that 28 days of treatment with the FGF21 analog LY2405319 decreases low-density lipoprotein cholesterol and triglycerides, increases high-density lipoprotein cholesterol and improves fasting insulin (48). These data provide a scenario whereby metabolic health might be improved via the manipulation of systemic FGF21.

183

184 B/ Effect of exercise on FGF21

185 Given the aforementioned possible role of FGF21 in mediating metabolic health, it is of
186 interest to identify ways in which FGF21 secretion can be altered. Exercise alters the
187 expression of FGF21 with initial investigations suggesting that FGF21 is a myokine (71, 72).
188 Indeed, transgenic mice (overexpressing Akt) characterized with increased muscle mass and
189 strength exhibited a significant increase in systemic FGF21 compared with littermate controls.
190 Moreover, in cultured skeletal muscle cells, FGF21 expression and secretion was regulated by
191 Akt transduction supporting the idea that FGF21 is a myokine (71). However, recent studies
192 have questioned this. Hansen et al. analysed the direct production of FGF21 using hepatic and
193 femoral vein and artery catheterization (59). They demonstrated, in healthy men, that FGF21
194 was secreted from the hepatosplanchnic bed but not in the leg during and after a prolonged
195 bout of endurance exercise. In line with these results, investigations involving rodents support
196 the contention that FGF21 is produced by liver. A single bout of endurance exercise
197 significantly increases hepatic *FGF21* mRNA expression, while the results are divergent in
198 skeletal muscle (59, 81, 97, 159). Interestingly, when healthy male subjects were infused with
199 glucagon and somatostatin to mimic exercise (6 ng.kg⁻¹.min⁻¹ and 100 ng.kg⁻¹.min⁻¹
200 respectively) splanchnic FGF21 levels were significantly increased compared to saline infusion
201 (59). Conversely, exercise with a pancreatic clamp (somatostatin, 100 ng.kg⁻¹.min⁻¹)
202 completely blunted the exercise-induced increase in plasma FGF21, suggesting a role for
203 pancreatic hormones in the regulation of hepatic FGF21 (60). In line with these results,
204 glucagon receptor knockout mice have a blunted induction of *FGF21* mRNA in the liver in
205 response to exercise (12). Also, resistance exercise, which elicits an increase in plasma
206 insulin, does not induce FGF21 release in the bloodstream (114). Moreover, circulating
207 FGF21 is also under the control of free fatty acids (FFA) levels during exercise in healthy

men (60, 81). Mechanistically, incubation of the FaO cell line with palmitic acid triggered FGF21 transcription through the concomitant action of the activating transcription factor 4 (ATF4) and peroxisome proliferator-activated receptor alpha (PPAR α). It could be hypothesized that exercise-induced lipolysis favours FGF21 production by the liver through an ATF4/PPAR α pathway. Thus, FGF21 production by the liver during exercise appears to be regulated by a synergetic action of glucagon to insulin ratio and FFA levels. A caveat is that all these experiments were mainly performed in healthy subjects. A recent study revealed that exercise-induced plasma FGF21 elevation was abolished in patients with T2D (60) suggesting that FGF21 production in response to acute exercise is altered in patients with metabolic disruption. While basal FGF21 was higher in T2D patients compared with healthy subjects (60), it appears that hyperinsulinemia or hepatic insulin-resistance would rather impair exercise-induced FGF21 secretion. Indeed, it has been reported that FGF21 secretion is lower in obese patients with hyperinsulinemia compared with healthy subjects (143). Interestingly, there was no difference in basal FGF21 concentrations between both groups, but the clear mechanism affecting FGF21 secretion during exercise in the context of metabolic disease remain to be elucidated.

Other studies have assessed the impact of chronic exercise on circulating levels of FGF21 in the context of metabolic disorders. In humans, the results seem controversial. Some studies support the idea that chronic exercise, combined or not with diet intervention, can significantly decrease circulating FGF21 in obese or elderly people (157, 158, 174) while others did not observe any effect in obese or diabetic patients (5, 15, 84). It is important to note that some methodological issues might explain these discrepancies. Firstly, these studies were performed in heterogeneous populations with respect to metabolic disruption. Also, FGF21 systemic levels are affected by various stimuli such as nutrient intake (98), fasting

status (38) or circadian rhythm (177) that were not specified in these studies. Finally, not all these studies examined changes in systemic levels of insulin or FFA, hepatic fat content, or cardiorespiratory fitness which are seemingly important factors affecting FGF21 levels (60, 157).

In a rodent model of T2D (OLETF), Fletcher et al. have investigated the effect of voluntary wheel running on FGF21 expression (43). The authors observed that active rats had a preserved hepatic mRNA and circulating FGF21 response compared to their sedentary littermates. Additionally, some studies in transgenic mice investigated whether FGF21 is necessary to mediate the effects of chronic exercise on improved energy metabolism. However, voluntary wheel running reduced adiposity, adipose tissue inflammation, hyperinsulinemia, and hepatic fatty acid content and oxidation in both FGF21 KO mice and their control littermates (44, 129). On the contrary, in mice fed with a high fat diet (HFD), voluntary wheel running did not improve hepatic triglyceride content and glucose tolerance but prevented weight and fat mass gain independently of genotype (97). The authors concluded that FGF21 KO mice exhibited an impaired adaptation to exercise training, including reduced AMP-activated protein kinase activity in skeletal muscle. Based on these findings, FGF21 may be necessary for the health-benefits associated with regular exercise under high fat, but not normal, dietary conditions. As the liver is the main source of FGF21 (101), further examinations in liver specific deletion models of FGF21 would help to better understand the cellular adaptations to physical activity.

To sum up, FGF21 plays a pleiotropic role in lipid and glucose metabolism and can improve metabolic-related disorders. It is now well accepted that exercise contributes to the prevention of chronic diseases, but the underlying mechanisms are not well understood. Interestingly, the

metabolic actions of FGF21 share those observed in response to exercise. Thus, the exercise-induced production of FGF21 by the liver might represent one of the cellular mechanisms involved in the metabolic adaptations to exercise. Also, FGF21 interacts with many tissues and its production during exercise might facilitate inter-organ crosstalk.

Fetuin-A

Fetuin-A is a 64 kDa glycoprotein known as an endogenous ligand for Toll-like receptor 4 (TLR4) and encoded by the *AHSG* gene (121). This receptor is expressed in several organs and more specifically in tissues involved in substrate metabolism such as the liver (54), adipose tissue (127) and skeletal muscle (47). Fetuin-A has also been shown to bind the β subunit of the insulin receptor (52).

A/ Fetuin A in metabolic disease

Much like FGF21, Fetuin-A has been proposed as a biomarker for metabolic diseases (122). For example, in a large cohort of 3170 community-living elderly individuals, a ten year follow up revealed that higher plasma Fetuin-A was associated with an increased incidence of T2D (70). More generally, it has been reported that circulating levels of Fetuin-A are positively correlated with impaired glucose tolerance, insulin resistance, T2D and liver fibrosis (119, 120, 152, 175), while an association with hepatic fat accumulation remains unclear (7). Significantly, several works assert that Fetuin-A might play a pivotal role in the pathogenesis of metabolic disorders. Firstly, data from transgenic mice demonstrate that Fetuin-A participates in the onset of metabolic dysfunction (106, 107). Indeed, Fetuin-A KO mice were protected from the deleterious effects of high fat diet with improved glucose clearance rate. This was associated with a higher insulin-stimulated phosphorylation of insulin receptor and the downstream signalling molecules MAPK and Akt in both liver and skeletal

muscle (107). Secondly, it has been reported that a single injection of fetuin-A inhibits insulin-stimulated insulin receptor autophosphorylation and IRS-1 phosphorylation in the liver and skeletal muscle of rats suggesting that fetuin-A may participate in the development of insulin resistance. Finally, *in vivo* and *in vitro* models of insulin resistance reinforce the idea that Fetuin-A is upregulated and released in the context of metabolic disruption. *In vivo*, it was observed that the expression of *Fetuin-A* mRNA in liver was increased by a high fat diet in rats (96). Also, F-box and WD repeat domain-containing 7 (FBXW7), an E3 ubiquitin protein ligase involved in Fetuin-A ubiquitination and degradation, is markedly downregulated in the liver of obese patients (181). *In vitro*, Takata and colleagues reported that glucose infusion increased Fetuin-A protein expression and *AHSG* transcription through the activation of ERK1/2 signalling in HepG2 cells (155). Also, palmitate incubation increased Fetuin-A protein expression and secretion through activation of NF- κ B in HepG2 cells and rat hepatocytes (33). When secreted, Fetuin-A represents an endogenous ligand for TLR4 through which FFA induces insulin resistance, macrophage infiltration and inflammation in adipocytes (121, 150). It is important to note that TLR4 KO mice have been shown to be protected from insulin resistance induced by lipid infusion or by HFD (139). Similarly, TLR4 activation in adipocytes resulted in insulin resistance (144). An effect of Fetuin-A has also been shown in the pancreas. While Fetuin-A promotes lipotoxicity of β -cells through a TLR4-signaling pathway (138), it also impairs glucose-induced insulin secretion in a TLR-4-independent manner (49). Finally, Fetuin-A may also promote insulin resistance by direct binding to the β subunit of insulin receptors, leading to decreased tyrosine kinase activity of the receptor (52, 105). Together, these results support that Fetuin-A affects insulin secretion and resistance, adipose tissue inflammation and thus may participate in the pathogenesis of metabolic disorders.

B/ Effect of exercise on Fetuin-A

There are only two published articles that have investigated the effect of a single bout of exercise on Fetuin-A. The results show that a 60min session of cycling/treadmill exercise (60% of VO_{2max}) does not modify circulating levels of Fetuin-A in both healthy and obese subjects (137). In obese individuals, serum phosphofetuin-A (Ser312) levels were immediately increased after a single bout of exercise (60-70% VO_{2max} expending 500 kcals) which decreased to baseline in 24 hours (104). Interestingly, glucose and insulin during OGTT were significantly decreased 24 hours after the session of exercise suggesting that exercise-induced lowering of Fetuin-A might participate in this acute health-benefit of exercise.

All other studies assessed the effect of chronic exercise on Fetuin-A. In rodents, Sakr et al. (2014) have shown that 16 weeks of swimming exercise in male Sprague Dawley rats suffering from metabolic syndrome significantly decreases Fetuin-A serum levels and improves HOMA-IR index (135). Similar findings were reported in human by Malin et al (2013) who studied the effect of 7 days of endurance training (60min at 85% HR_{max}) on plasma Fetuin-A concentrations in obese patients with NAFLD (99). The authors observed a significant decrease in circulating Fetuin-A which was positively correlated with a reduced insulin resistance index and improved glucose tolerance. Later, the same team revealed that 12 weeks of endurance training induced a significant decrease of plasma Fetuin-A, which correlated with a decrease in hepatic, but not skeletal muscle or adipose insulin resistance (100). Interestingly, the effect of exercise on the decrease of Fetuin-A levels was not associated with a change in hepatic triglyceride content (99). One plausible explanation would be that exercise-induced changes in fetuin-A may relate to changes in blood lipids rather than liver fat content. Indeed, Lee and colleagues reported that the decrease in plasma fetuin-A and FFA interacted to improve glucose infusion rate in sedentary and overweight disglycemic

men in response to 12 weeks of concurrent training (90). Importantly, the decrease in plasma concentration of fetuin-A predicted changes in gene expression related to inflammatory TLR-signalling in macrophages in adipose tissue.

It is now well established that chronic exercise is beneficial for diseases associated with low-grade inflammation such as obesity, T2D, NAFLD or NASH (18, 75, 76). Altogether, we could hypothesize that exercise-induced lowering of fetuin-A through the downregulation of TLR4 pathway is one mechanism that participates in this anti-inflammatory process. Also, these studies suggest that regular exercise improves whole body and liver insulin sensitivity in patients with metabolic disease by decreasing circulating Fetuin-A levels. Thus, if exercise regulates Fetuin A expression, this might thus be one mechanism by which physical activity can influence the development of metabolic disease. One major limitation is that we are lacking studies examining the effect of a single bout of exercise on hepato-splanchnic production of fetuin-A. Also, it would be interesting to investigate the cellular modifications of the Fetuin-A signalling pathway in the liver in response to exercise.

Angiopoietin-like protein 4 (ANGPTL4)

Angiopoietin like protein 4 (ANGPTL4) a 45–65 kDa glycosylated and secreted protein which belongs to the angiopoietin-like gene family. *ANGPTL4* mRNA is expressed in liver but also in adipose tissue and to a lesser extent in skeletal muscle (78).

A/ ANGPTL4 in metabolic disease

Little is known about the determinants of plasma ANGPTL4 and its clinical relevancy in metabolic disorders. So far, a clear, positive relationship with plasma FFA has been shown (78, 83, 132). Furthermore, obese subjects generally have higher levels of plasma ANGPTL4

while an association with OGTT- and hyperinsulinemic-euglycemic clamp-derived indexes of insulin sensitivity are not clear (146). It is well established that ANGPTL4 participates in the regulation of lipid metabolism via the stimulation of lipolysis in adipocytes in a fasting state (53) and the inhibition of lipoprotein lipase (LPL) activity (88). LPL is an enzyme responsible for the hydrolysis of the triglyceride (TG) core of circulating TG-rich lipoproteins resulting in FFA which can be either stored or oxidized. Thus, overexpression of ANGPTL4 in mice resulted in a dramatic increase in circulating triglycerides and cholesterol, associated with a decrease in LPL activity, compared with wild-type littermates (85). Concerning glucose metabolism, the role of ANGPTL4 is unclear. Overexpression of Angptl4 by adenovirus improved glucose tolerance in mice compared with control, and reduced hepatic glucose production in rat hepatocytes (171). On the contrary, hyperinsulinemic-euglycemic clamp analyses revealed that whole-body transgenic overexpression of ANGPTL4 causes impaired glucose utilisation and insulin resistance, and higher insulin-mediated suppression of glucose production in the liver (94). Recently, Janssen and colleagues investigated the effect of whole-body deletion of ANGPTL4 on glucose homeostasis and metabolic function using a diet-induced obesity model (73). The authors observed that ANGPTL4^{-/-} mice exhibited elevated fat mass, visceral fat mass and inflammation but, interestingly, improved glucose tolerance compared with wild type controls. Specific adipose tissue deletion of ANGPTL4 also resulted in improved glucose metabolism, associated with decreased ectopic lipid deposition in the liver and skeletal muscle (8). Overall, ANGPTL4 seems to display a dichotomous effect on lipid and glucose metabolism.

B/ Effect of exercise on ANGPTL4

Kersten and colleagues (2009) first reported that endurance cycling exercise (50% VO_{2max} for 2 h) increased ANGPTL4 circulating levels in fasted but not fed young healthy males (78).

383 Lately, micro-array analysis of *vastus lateralis* samples following a single bout of one-legged
384 cycle exercise (60min at 50% of maximum workload (W_{max})) revealed a significant increase
385 in *ANGPTL4* mRNA, interestingly in both legs with a more pronounced elevation in the non-
386 exercising limb (21). To better understand this difference and the role of ANGPTL4 in
387 exercise-induced metabolic adaptations, Catoire and colleagues repeated this one leg exercise
388 protocol to bring to light the regulatory mechanism (20). The authors revealed that induction
389 of ANGPTL4 in non-exercising muscle is mediated by elevated plasma free fatty acids via
390 PPAR δ , presumably leading to prevent fat overload and provide fatty acids to the active
391 skeletal muscle. However, it is unclear whether the increase in circulating ANGPTL4 levels is
392 triggered by an increased mRNA and production of ANGPTL4 from skeletal muscle or
393 whether other tissues contribute as well. Recently, an elegant investigation from Ingerslev and
394 colleagues depicted the mechanism of ANGPTL4 production in response to exercise (68). By
395 assessing arterial-to-venous differences over the leg and the hepato-splanchnic bed, the
396 authors revealed that the increase in plasma ANGPTL4 in exercising humans is liver-derived
397 with no contribution of the exercising muscles. Moreover, when exercise was performed
398 under pancreatic clamp to inhibit the increase in glucagon-to-insulin ratio and FFA,
399 ANGPTL4 production was blunted. This suggests that glucagon-to-insulin ratio and FFA
400 plays a pivotal role in ANGPTL4 production. *In vitro*, hormonal infusions revealed that the
401 glucagon-to-insulin ratio through the activation of the cAMP-PKA pathway triggered
402 *ANGPTL4* mRNA production in hepatocytes (68). Together, these data suggest that
403 ANGPTL4 is an exercise-induced hepatokine and that the skeletal muscle is not involved in
404 the increase of the plasma concentration. Notwithstanding, ANGPTL4 production by the
405 skeletal muscle during exercise may have an autocrine function (20, 146). Furthermore, while
406 a single bout of endurance exercise (60min at 14m.min⁻¹ and 14° inclination) increased
407 mRNA expression of *ANGPTL4* in liver of mice (65), it is unclear whether hepatocytes are

408 responsible for the increase of the serum protein level in response to exercise in this rodent
409 model. Also, Norheim and colleagues observed a significant increase in serum concentration
410 of ANGPTL4 in response to 60min of cycling (70% of VO₂max) which was even more
411 pronounced in dysglycemic subjects compared to controls (116).

412
413 Regarding chronic exercise, little is known about the impact of endurance training on
414 circulating ANGPTL4. In healthy humans, Catoire et al. (2014) observed that 2 weeks (a
415 session of 45 min, 3-min intervals at 70% and 35% W_{max} alternated with a session of 120
416 min at 50% W_{max}) or 12 weeks of endurance training (three times per week for 47.5 ± 2.5
417 min at 40% VO_{2max}) did not alter circulating ANGPTL4 (20). In obese patients, it has been
418 shown that 6 months of endurance training (3 times per week for 60-75 min at 70% of heart
419 rate (HR) reserve) resulted in a significant weight loss and an increase of serum ANGPTL4
420 (31).

421
422 Physical activity triggers short- and long-term adaptations to supply the energetic demands of
423 the body. Lipid metabolism is one of the key components and multiple mechanisms underpin
424 the adaptive responses to acute and chronic exercise. We describe here work suggesting that
425 ANGPTL4 regulates LPL activity and thus, plays a pivotal role in lipid metabolism. As
426 ANGPTL4 is an exercise-induced hepatokine, this mechanism could participate in the
427 adaptation of lipid metabolism to physical activity. However, it is now necessary to decipher
428 whether production of ANGPTL4 during exercise participates in the health-benefits of
429 physical activity to prevent and treat metabolic disease. Finally, as ANGPTL4 appears to play
430 an important role in skeletal muscle lipid metabolism (20), this hepatokine might participate
431 in exercise-induced inter-organ crosstalk between the liver and the skeletal muscle.

Follistatin (Fst)

Fst is a glycosylated plasma protein, which is a member of the TGF β superfamily. Fst was first described for its role in reproduction (86), but is also implicated in the regulation of the skeletal muscle mass (134). Recently, it was reported that Fst is highly expressed in the liver but also in skeletal muscle and white and brown adipose tissues (17, 57). There are two Fst isoforms: Fst 288 and Fst 315 (140).

A/ Fst in metabolic disease

It is thought that Fst levels are increased in patients with T2D, NAFLD and NASH compared with control subjects and that they correlate positively with HbA1c, fasting blood glucose, and impaired glucose tolerance (58, 60, 176). Also, Polyzos and colleagues assessed the circulating levels of Fst in lean and obese subjects and patients with NAFLD or NASH (128). There was no difference between subjects but Fst levels were associated with NASH within NAFLD patients. The authors suggested that Fst may underlie the progression from NAFLD to NASH (128). Finally, recent findings reported that bariatric surgery significantly decreased Fst and this correlates with improved HbA1c in obese patients with diabetes (160). In line with these observations, *in vivo* and *in vitro* investigations support the idea that Fst plays a pivotal role in glucose metabolism. It has been shown that Fst participates in systemic metabolic dysregulation by hepatic FoxO1 activity (160). Also, during HFD, overexpression of Fst315 by adenovirus in mice impaired the glycaemic response to OGTT compared with control mice (160). Interestingly, *Fst315*-KO mice exhibit steatosis while hepatic insulin signalling, as assessed by phospho-Akt in response to insulin injection, was improved (163). Recently, an elegant study from Tao and colleagues suggested that Fst targets hepatic glucose production (160). In a mouse model of insulin resistance (LDKO), silencing the hepatic Fst allele restored glucose tolerance and insulin levels compared with control LDKO. Also,

hyperinsulinemic-euglycemic clamps revealed an improvement in insulin sensitivity, through an increase in Akt signalling in white adipose tissue and a decrease in hepatic glucose production. Thus, it appears that Fst is a hepatokine which participates in the development of metabolic disorders. However, further clinical studies are needed to clearly establish the role of Fst in metabolic disorders. Also, it is important to note that Fst seems to have opposite functions depending on the tissues. For example, overexpression of Fst in pancreatic β -cells improved fasting blood glucose in db/db mice (180) suggesting a complex role of Fst in metabolism.

B/ Effect of exercise on Fst

Fst was studied in the area of exercise because of its role in regulating skeletal muscle hypertrophy by antagonizing myostatin (32, 34, 51). Recent findings reported that Fst is released in the bloodstream in response to an acute bout of exercise. A first study performed by Hansen and colleagues revealed that 3h of cycling at 50% of VO_{2max} increased circulating levels of Fst but not *Fst* mRNA content in the vastus lateralis of healthy subjects (57). When the authors assessed the response of 1h of swimming in mice in several tissues, they observed a marked increase of mRNA content and protein level of Fst in the liver but not in skeletal muscle. This prompted the authors to determine the source of Fst during exercise in humans using liver vein catheterization (61). A significant increase in Fst in both hepatic vein and artery in response to 2h of cycling at 60% of VO_{2max} was observed. More importantly, arterial-to-venous differences was negative during the exercise session demonstrating a constant hepatic secretion of Fst from the splanchnic bed. This secretion can partly be explained by an increase in glucagon to insulin ratio during exercise (60). Indeed, combined somatostatin-glucagon infusion increased plasma Fst while its secretion in response to exercise during a pancreatic clamp was partially blunted in humans (60, 61). This hypothesis

was reinforced by *in vitro* investigations that revealed that glucagon increases, and insulin inhibits Fst production through the secondary messenger cAMP in hepatocytes (61). It is important to note that Fst secretion during exercise is impaired in patients with T2D (60) but not in obese subjects (137). Together, these studies suggest that an acute bout of exercise leads to Fst liver secretion.

While the acute regulation of Fst by exercise is partially characterised, the relationship between chronic exercise and Fst has not been extensively studied. It has been reported that resistance training is associated with an increase in circulating Fst in elderly overweight women (66). Also, high-intensity interval training (HIIT) increase Fst levels in sedentary but not life-long active elderly subjects (36). Regarding hepatic Fst, one study observed that 4 weeks of swimming training decreased similarly mRNA content of *Fst* in both lean and obese rats when compared with controls (141).

Regular physical activity is well known to promote glucose control and insulin sensitivity. We summarize here that Fst may participate in the regulation of these processes and in the development of metabolic disorders. Evidence suggests that Fst is an exercise-induced hepatokine, but little is known about its long-term adaptation to regular exercise. However, due to its biological properties mentioned above, it could be speculated that Fst participates in the cellular adaptation to exercise and to metabolic disease prevention. Also, Fst is involved in skeletal muscle mass hypertrophy and in β -cell function, and could mediate exercise-induced inter-organ crosstalk.

Methodological limitations and future directions.

In this review, we aimed to summarize the current literature regarding some proposed hepatokines involved in metabolic functions that are secreted in response to an acute session

508 of exercise and their regulation in response to training. While results are promising to better
509 understand the cellular and molecular adaptations to exercise, several challenges need to be
510 overcome. From a methodological point of view, key points need to be addressed before
511 considering a protein as an exercise-induced hepatokine that participates in the health-
512 promoting benefits of exercise. Firstly, clearly demonstrating a protein is secreted by the liver
513 is technically challenging and we have mentioned how researchers have used arterial-venous
514 difference analyses to overcome this (59, 61, 68). Secondly, determining the key function of a
515 protein released from the liver is difficult. However, the generation of hepatocyte-specific
516 gene knockout mouse models is a useful approach. For example, employing the Cre/Lox
517 system, Markan and colleagues generated a model of mice lacking FGF21 specifically in the
518 liver (101) which could be used to assess if FGF21 is necessary for the beneficial metabolic
519 adaptations to exercise. Similarly, liver specific adenoviral overexpression of FGF21 (93) could
520 help clarify the role of FGF21 in training.

521 Another challenge is to discover new exercise-responsive hepatokines that are released from
522 the liver to influence whole-body glucose or lipid homeostasis. To do so, deep proteomic
523 analyses associated with mass spectrometry may allow identification of new hepatokine
524 candidates. Recently, Meex and colleagues suggested that purified hepatocytes can secrete
525 more than 500 proteins with 114 differentially expressed under steatotic conditions (108). A
526 similar approach could be envisaged to test the effect of exercise upon the liver secretome.
527 When identified, specific attention should be paid on the cellular mechanisms involved in
528 hepatokine expression, secretion and action. For instance, there are two proposed mechanisms
529 that trigger hepatokine release in response to exercise: glucagon to insulin ratio and FFA
530 levels (**Fig. 1**). The hormonal changes during exercise generally occur to ensure
531 cardiovascular adjustments, energy substrate disposal and/or hydration (55). Thus, it could be
532 hypothesized that hepatokine secretion acts as a conduit for the adaptation to exercise.

Regarding their actions, we reported here that hepatokines are secreted in to the bloodstream in response to a single bout of endurance exercise. As hepatokines can interact with other tissues, we can speculate that exercise-induced secreted protein from the liver participates in inter-tissue crosstalk.

It is well accepted that whole body homeostasis is influenced differently by exercise depending on its modality and the conditions in which it is performed. There exists a broad range of resistance or aerobic exercises such as classical moderate intensity continuous training (MICT), as well as the more recently proposed HIIT programs or sprint interval training (SIT) (77). Interestingly, it appears that short period HIIT training is well tolerated by patients and has a pronounced impact on glycemic control in patients with T2D (46). Thus, further studies are warranted to determine the optimal modalities of exercise that trigger hepatokine secretion to help the clinician to prescribe physical activity. In addition, nutritional status (fed vs fasted) or strategies (*ie* post exercise carbohydrate consumption) should be investigated in the context of hepatokine secretion. For example, exercise-induced plasma ANGPTL4 increases were blunted in the fed compared with the fasted state (78).

Finally, the aforementioned studies regarding exercise and hepatokines were performed in a broad range of subjects with respect to sex, age and metabolic disruptions. Thus, clinical studies are warranted in large cohort of patients with a long term follow up to decipher the contribution of hepatokines in metabolic adaptations to physical activity and, ultimately, improve the management of obesity, insulin-resistance, T2D, NAFLD and NASH through adapted training programs.

Review criteria

Searches for original articles or abstracts published between 1990 and December 2018 focusing on hepatokines in metabolic diseases and in exercise were performed in MEDLINE

and PubMed. The search terms used were “liver”, “exercise”, “physical activity”,
“hepatokine”, “fetuin-A”, “follistatin”, “fibroblast growth factor 21”, “angiopoietin-like
protein 4”, “obesity”, “inflammation”, “type 2 diabetes”, “nonalcoholic fatty liver disease”
and “insulin resistance”. All articles identified were in the English-language. We apologise in
advance to any researchers whose relevant work may have been missed using this criteria.

Declaration of interest statement

Pascal Sirvent is employed by Valbiotis S.A.S.

- 567 1. **Adams LA, Anstee QM, Tilg H, Targher G.** Non-Alcoholic fatty liver disease and its relationship
568 with cardiovascular disease and other extrahepatic diseases. *Gut* 66: 1138–1153, 2017.
- 569 2. **Alamuddin N, Wadden TA.** Behavioral Treatment of the Patient with Obesity. *Endocrinol*
570 *Metab Clin North Am* 45: 565–80, 2016.
- 571 3. **Alkhoury N, Tamimi TAR, Yerian L, Lopez R, Zein NN, Feldstein AE.** The inflamed liver and
572 atherosclerosis: A Link between histologic severity of nonalcoholic fatty liver disease and
573 increased cardiovascular risk. *Dig Dis Sci* 55: 2644–2650, 2010.
- 574 4. **Andersen B, Straarup EM, Heppner KM, Takahashi DL, Raffaele V, Dissen GA, Lewandowski**
575 **K, Bödvarsdóttir TB, Raun K, Grove KL, Kievit P.** FGF21 decreases body weight without
576 reducing food intake or bone mineral density in high-fat fed obese rhesus macaque monkeys.
577 *Int J Obes* 42: 1151–1160, 2018.
- 578 5. **Andersen TR, Schmidt JF, Thomassen M, Hornstrup T, Frandsen U, Randers MB, Hansen PR,**
579 **Krustrup P, Bangsbo J.** A preliminary study: effects of football training on glucose control,
580 body composition, and performance in men with type 2 diabetes. *Scand J Med Sci Sports* 24
581 Suppl 1: 43–56, 2014.
- 582 6. **Archer AE, Rogers RS, Von Schulze AT, Wheatley JL, Morris EM, McCoin CS, Thyfault JP,**
583 **Geiger PC.** Heat shock protein 72 regulates hepatic lipid accumulation. *Am J Physiol Integr*
584 *Comp Physiol* 315: R696–R707, 2018.
- 585 7. **Aroner SA, Mukamal KJ, St-Jules DE, Budoff MJ, Katz R, Criqui MH, Allison MA, De Boer IH,**
586 **Siscovick DS, Ix JH, Jensen MK.** Fetuin-A and risk of diabetes independent of liver fat content:
587 The multi-ethnic study of atherosclerosis. *Am J Epidemiol* 185: 54–64, 2017.
- 588 8. **Aryal B, Singh AK, Zhang X, Varela L, Rotllan N, Goedeke L, Chaube B, Camporez J-P, Vatner**
589 **DF, Horvath TL, Shulman GI, Suárez Y, Fernández-Hernando C.** Absence of ANGPTL4 in
590 adipose tissue improves glucose tolerance and attenuates atherogenesis. *JCI Insight* 3, 2018.
- 591 9. **Auberger P, Falquerho L, Contreras JO, Pages G, Cam G Le, Rossi B, Cam A Le.**
592 Characterization of a natural inhibitor of the insulin receptor tyrosine kinase: cDNA cloning,
593 purification, and anti-mitogenic activity. *Cell* 58: 631–640, 1989.
- 594 10. **Awazawa M, Gabel P, Tsousidou E, Nolte H, Krüger M, Schmitz J, Ackermann PJ, Brandt C,**
595 **Altmüller J, Motameny S, Wunderlich FT, Kornfeld JW, Blüher M, Brüning JC.** A microRNA
596 screen reveals that elevated hepatic ectodysplasin A expression contributes to obesity-
597 induced insulin resistance in skeletal muscle. *Nat Med* 23: 1466–1473, 2017.
- 598 11. **Badman MK, Koester A, Flier JS, Kharitonov A, Maratos-Flier E.** Fibroblast growth factor
599 21-deficient mice demonstrate impaired adaptation to ketosis. *Endocrinology* 150: 4931–40,
600 2009.
- 601 12. **Berglund ED, Kang L, Lee-Young RS, Hasenour CM, Lustig DG, Lynes SE, Donahue EP, Swift LL,**
602 **Charron MJ, Wasserman DH.** Glucagon and lipid interactions in the regulation of hepatic
603 AMPK signaling and expression of PPARalpha and FGF21 transcripts in vivo. *Am J Physiol*
604 *Endocrinol Metab* 299: E607–E614, 2010.
- 605 13. **Berglund ED, Li CY, Bina HA, Lynes SE, Michael MD, Shanafelt AB, Kharitonov A,**
606 **Wasserman DH.** Fibroblast growth factor 21 controls glycemia via regulation of hepatic
607 glucose flux and insulin sensitivity. *Endocrinology* 150: 4084–4093, 2009.
- 608 14. **Berti L, Irmeler M, Zdichavsky M, Meile T, Böhm A, Stefan N, Fritsche A, Beckers J,**

- 609 **Königsrainer A, Häring HU, de Angelis MH, Staiger H.** Fibroblast growth factor 21 is elevated
610 in metabolically unhealthy obesity and affects lipid deposition, adipogenesis, and adipokine
611 secretion of human abdominal subcutaneous adipocytes. *Mol Metab* 4: 519–527, 2015.
- 612 15. **Besse-Patin A, Montastier E, Vinel C, Castan-Laurell I, Louche K, Dray C, Daviaud D, Mir L,**
613 **Marques M-A, Thalamas C, Valet P, Langin D, Moro C, Viguerie N.** Effect of endurance
614 training on skeletal muscle myokine expression in obese men: identification of apelin as a
615 novel myokine. *Int J Obes (Lond)* 38: 707–13, 2014.
- 616 16. **Booth FW, Roberts CK, Laye MJ.** Lack of exercise is a major cause of chronic diseases. *Compr*
617 *Physiol* 2: 1143–211, 2012.
- 618 17. **Braga M, Reddy ST, Vergnes L, Pervin S, Grijalva V, Stout D, David J, Li X, Tomasian V, Reid**
619 **CB, Norris KC, Devaskar SU, Reue K, Singh R.** Follistatin promotes adipocyte differentiation,
620 browning, and energy metabolism. *J Lipid Res* 55: 375–384, 2014.
- 621 18. **Bruun JM.** Diet and exercise reduce low-grade inflammation and macrophage infiltration in
622 adipose tissue but not in skeletal muscle in severely obese subjects. *AJP Endocrinol Metab*
623 290: E961–E967, 2006.
- 624 19. **Camporez JPG, Asrih M, Zhang D, Kahn M, Samuel VT, Jurczak MJ, Jornayvaz FR.** Hepatic
625 insulin resistance and increased hepatic glucose production in mice lacking Fgf21. *J Endocrinol*
626 226: 207–17, 2015.
- 627 20. **Catoire M, Alex S, Paraskevopoulos N, Mattijssen F, Evers-van Gogh I, Schaart G, Jeppesen J,**
628 **Kneppers A, Mensink M, Voshol PJ, Olivecrona G, Tan NS, Hesselink MKC, Berbée JF, Rensen**
629 **PCN, Kalkhoven E, Schrauwen P, Kersten S.** Fatty acid-inducible ANGPTL4 governs lipid
630 metabolic response to exercise. *Proc Natl Acad Sci U S A* 111: E1043–52, 2014.
- 631 21. **Catoire M, Mensink M, Boekschoten M V., Hangelbroek R, Müller M, Schrauwen P, Kersten**
632 **S.** Pronounced Effects of Acute Endurance Exercise on Gene Expression in Resting and
633 Exercising Human Skeletal Muscle. *PLoS One* 7: e51066, 2012.
- 634 22. **Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, Harrison SA, Brunt EM,**
635 **Sanyal AJ.** The diagnosis and management of nonalcoholic fatty liver disease: Practice
636 guidance from the American Association for the Study of Liver Diseases. *Hepatology* 67: 328–
637 357, 2018.
- 638 23. **Chanda D, Li J, Oligschlaeger Y, Jeurissen MLJ, Houben T, Walenbergh SMA, Shiri-Sverdlov R,**
639 **Neumann D.** MSP is a negative regulator of inflammation and lipogenesis in ex vivo models of
640 non-alcoholic steatohepatitis. *Exp Mol Med* 48: e258–e258, 2016.
- 641 24. **Chavez AO, Molina-Carrion M, Abdul-Ghani MA, Folli F, DeFronzo RA, Tripathy D.** Circulating
642 Fibroblast Growth Factor-21 Is Elevated in Impaired Glucose Tolerance and Type 2 Diabetes
643 and Correlates With Muscle and Hepatic Insulin Resistance. *Diabetes Care* 32, 2009.
- 644 25. **Chen L, Magliano DJ, Zimmet PZ.** The worldwide epidemiology of type 2 diabetes mellitus—
645 present and future perspectives. *Nat Rev Endocrinol* 8: 228–236, 2011.
- 646 26. **Chen Z, Ding L, Yang W, Wang J, Chen L, Chang Y, Geng B, Cui Q, Guan Y, Yang J.** Hepatic
647 Activation of the FAM3C-HSF1-CaM Pathway Attenuates Hyperglycemia of Obese Diabetic
648 Mice. *Diabetes* 66: 1185–1197, 2017.
- 649 27. **Choi HY, Hwang SY, Lee CH, Hong HC, Yang SJ, Yoo HJ, Seo JA, Kim SG, Kim NH, Baik SH, Choi**
650 **DS, Choi KM.** Increased Selenoprotein P Levels in Subjects with Visceral Obesity and

- 651 Nonalcoholic Fatty Liver Disease. *Diabetes Metab J* 37: 63, 2013.
- 652 28. **Chung J, Nguyen A-K, Henstridge DC, Holmes AG, Chan MHS, Mesa JL, Lancaster GI,**
653 **Southgate RJ, Bruce CR, Duffy SJ, Horvath I, Mestril R, Watt MJ, Hooper PL, Kingwell BA,**
654 **Vigh L, Hevener A, Febbraio MA.** HSP72 protects against obesity-induced insulin resistance.
655 *Proc Natl Acad Sci U S A* 105: 1739–44, 2008.
- 656 29. **Coskun T, Bina HA, Schneider MA, Dunbar JD, Hu CC, Chen Y, Moller DE, Kharitonov A.**
657 Fibroblast growth factor 21 corrects obesity in mice. *Endocrinology* 149: 6018–6027, 2008.
- 658 30. **Crescenzo R, Bianco F, Mazzoli A, Giacco A, Liverini G, Iossa S.** A possible link between
659 hepatic mitochondrial dysfunction and diet-induced insulin resistance. *Eur J Nutr* 55: 1–6,
660 2016.
- 661 31. **Cullberg KB, Christiansen T, Paulsen SK, Bruun JM, Pedersen SB, Richelsen B.** Effect of weight
662 loss and exercise on angiogenic factors in the circulation and in adipose tissue in obese
663 subjects. *Obesity (Silver Spring)* 21: 454–60, 2013.
- 664 32. **Dalbo VJ, Roberts MD, Sunderland KL, Poole CN, Stout JR, Beck TW, Bemben M, Kersick**
665 **CM.** Acute Loading and Aging Effects on Myostatin Pathway Biomarkers in Human Skeletal
666 Muscle After Three Sequential Bouts of Resistance Exercise. *Journals Gerontol Ser A Biol Sci*
667 *Med Sci* 66A: 855–865, 2011.
- 668 33. **Dasgupta S, Bhattacharya S, Biswas A, Majumdar SS, Mukhopadhyay S, Ray S, Bhattacharya**
669 **S.** NF- κ B mediates lipid-induced fetuin-A expression in hepatocytes that impairs adipocyte
670 function effecting insulin resistance. *Biochem J* 429: 451–462, 2010.
- 671 34. **Dieli-Conwright CM, Spektor TM, Rice JC, Sattler FR, Schroeder ET.** Influence of hormone
672 replacement therapy on eccentric exercise induced myogenic gene expression in
673 postmenopausal women. *J Appl Physiol* 107: 1381–1388, 2009.
- 674 35. **Eckardt K, Görgens SW, Raschke S, Eckel J.** Myokines in insulin resistance and type 2 diabetes.
675 *Diabetologia* 57: 1087–99, 2014.
- 676 36. **Elliott BT, Herbert P, Sculthorpe N, Grace FM, Stratton D, Hayes LD, Elliott CBT.** Lifelong
677 exercise, but not short-term high-intensity interval training, increases GDF11, a marker of
678 successful aging: a preliminary investigation. *Physiol Rep* 5, 2017.
- 679 37. **Fabbrini E, Magkos F, Mohammed BS, Pietka T, Abumrad NA, Patterson BW, Okunade A,**
680 **Klein S.** Intrahepatic fat, not visceral fat, is linked with metabolic complications of obesity.
681 *Proc Natl Acad Sci* 106: 15430–15435, 2009.
- 682 38. **Fazeli PK, Lun M, Kim SM, Bredella MA, Wright S, Zhang Y, Lee H, Catana C, Klibanski A,**
683 **Patwari P, Steinhauser ML.** FGF21 and the late adaptive response to starvation in humans. *J*
684 *Clin Invest* 125: 4601–11, 2015.
- 685 39. **Febbraio MA, Hiscock N, Sacchetti M, Fischer CP, Pedersen BK.** Interleukin-6 is a novel factor
686 mediating glucose homeostasis during skeletal muscle contraction. *Diabetes* 53: 1643–8,
687 2004.
- 688 40. **Febbraio MA, Mesa JL, Chung J, Steensberg A, Keller C, Nielsen HB, Krstrup P, Ott P, Secher**
689 **NH, Pedersen BK.** Glucose ingestion attenuates the exercise-induced increase in circulating
690 heat shock protein 72 and heat shock protein 60 in humans. [Online]. *Cell Stress Chaperones*
691 9: 390–6, 2004. <http://www.ncbi.nlm.nih.gov/pubmed/15633297> [16 Feb. 2019].

- 692 41. **Febbraio MA, Ott P, Nielsen HB, Steensberg A, Keller C, Krstrup P, Secher NH, Pedersen BK.**
693 Exercise induces hepatosplanchnic release of heat shock protein 72 in humans. *J Physiol* 544:
694 957–62, 2002.
- 695 42. **Fisher ffolliott M, Kim MS, Doridot L, Cunniff JC, Parker TS, Levine DM, Hellerstein MK,**
696 **Hudgins LC, Maratos-Flier E, Herman MA.** A critical role for ChREBP-mediated FGF21
697 secretion in hepatic fructose metabolism. *Mol Metab* 6: 14–21, 2017.
- 698 43. **Fletcher J a, Meers GM, Laughlin MH, Ibdah J a, Thyfault JP, Rector RS.** Modulating fibroblast
699 growth factor 21 in hyperphagic OLETF rats with daily exercise and caloric restriction. *Appl*
700 *Physiol Nutr Metab* 37: 1054–62, 2012.
- 701 44. **Fletcher JA, Linden MA, Sheldon RD, Meers GM, Morris EM, Butterfield A, Perfield JW,**
702 **Thyfault JP, Rector RS.** Fibroblast growth factor 21 and exercise-induced hepatic
703 mitochondrial adaptations. *Am J Physiol Gastrointest Liver Physiol* 310: G832-43, 2016.
- 704 45. **Fon Tacer K, Bookout AL, Ding X, Kurosu H, John GB, Wang L, Goetz R, Mohammadi M, Kuro-**
705 **o M, Mangelsdorf DJ, Kliewer SA.** Research Resource: Comprehensive Expression Atlas of the
706 Fibroblast Growth Factor System in Adult Mouse. *Mol Endocrinol* 24: 2050–2064, 2010.
- 707 46. **Francois ME, Little JP.** Effectiveness and safety of high-intensity interval training in patients
708 with type 2 diabetes. *Diabetes Spectr* 28: 39–44, 2015.
- 709 47. **Frisard MI, McMillan RP, Marchand J, Wahlberg KA, Wu Y, Voelker KA, Heilbronn L, Haynie**
710 **K, Muoio B, Li L, Hulver MW.** Toll-like receptor 4 modulates skeletal muscle substrate
711 metabolism. *Am J Physiol Endocrinol Metab* 298: E988-98, 2010.
- 712 48. **Gaich G, Chien JY, Fu H, Glass LC, Deeg MA, Holland WL, Kharitonov A, Bumol T, Schilske**
713 **HK, Moller DE.** The effects of LY2405319, an FGF21 Analog, in obese human subjects with
714 type 2 diabetes. *Cell Metab* 18: 333–340, 2013.
- 715 49. **Gerst F, Wagner R, Kaiser G, Panse M, Heni M, Machann J, Bongers MN, Sartorius T, Sipos B,**
716 **Fend F, Thiel C, Nadalin S, Königsrainer A, Stefan N, Fritsche A, Häring HU, Ullrich S, Siegel-**
717 **Axel D.** Metabolic crosstalk between fatty pancreas and fatty liver: effects on local
718 inflammation and insulin secretion. *Diabetologia* 60: 2240–2251, 2017.
- 719 50. **Ghorpade DS, Ozcan L, Zheng Z, Nicoloso SM, Shen Y, Chen E, Blüher M, Czech MP, Tabas I.**
720 Hepatocyte-secreted DPP4 in obesity promotes adipose inflammation and insulin resistance.
721 *Nature* 555: 673–677, 2018.
- 722 51. **Gilson H, Schakman O, Kalista S, Lause P, Tsuchida K, Thissen J-P.** Follistatin induces muscle
723 hypertrophy through satellite cell proliferation and inhibition of both myostatin and activin.
724 *Am J Physiol Metab* 297: E157–E164, 2009.
- 725 52. **Goustin AS, Derar N, Abou-Samra AB.** Ahsg-fetuin blocks the metabolic arm of insulin action
726 through its interaction with the 95-kD β -subunit of the insulin receptor. *Cell Signal* 25: 981–
727 988, 2013.
- 728 53. **Gray NE, Lam LN, Yang K, Zhou AY, Koliwad S, Wang J-C.** Angiopoietin-like 4 (Angptl4) Protein
729 Is a Physiological Mediator of Intracellular Lipolysis in Murine Adipocytes. *J Biol Chem* 287:
730 8444–8456, 2012.
- 731 54. **Guo J, Friedman SL.** Toll-like receptor 4 signaling in liver injury and hepatic fibrogenesis.
732 *Fibrogenesis Tissue Repair* 3: 21, 2010.

- 733 55. **Hackney AC, Lane AR.** Exercise and the Regulation of Endocrine Hormones. In: *Progress in*
734 *molecular biology and translational science*. 2015, p. 293–311.
- 735 56. **Hakuno D, Kimura M, Ito S, Satoh J, Nakashima Y, Horie T, Kuwabara Y, Nishiga M, Ide Y,**
736 **Baba O, Nishi H, Nakao T, Nishino T, Nakazeki F, Koyama S, Hanada R, Randolph RR, Endo J,**
737 **Kimura T, Ono K.** Hepatokine α 1-Microglobulin Signaling Exacerbates Inflammation and
738 Disturbs Fibrotic Repair in Mouse Myocardial Infarction. *Sci Rep* 8: 16749, 2018.
- 739 57. **Hansen J, Brandt C, Nielsen AR, Hojman P, Whitham M, Febbraio MA, Pedersen BK,**
740 **Plomgaard P.** Exercise Induces a Marked Increase in Plasma Follistatin: Evidence That
741 Follistatin Is a Contraction-Induced Hepatokine. *Endocrinology* 152: 164–171, 2011.
- 742 58. **Hansen J, Rinnov A, Krogh-Madsen R, Fischer CP, Andreasen AS, Berg RMG, Møller K,**
743 **Pedersen BK, Plomgaard P.** Plasma follistatin is elevated in patients with type 2 diabetes:
744 relationship to hyperglycemia, hyperinsulinemia, and systemic low-grade inflammation.
745 *Diabetes Metab Res Rev* 29: 463–472, 2013.
- 746 59. **Hansen JS, Clemmesen JO, Secher NH, Hoene M, Drescher A, Weigert C, Pedersen BK,**
747 **Plomgaard P.** Glucagon-to-insulin ratio is pivotal for splanchnic regulation of FGF-21 in
748 humans. *Mol Metab* 4: 551–60, 2015.
- 749 60. **Hansen JS, Pedersen BK, Xu G, Lehmann R, Weigert C, Plomgaard P.** Exercise-Induced
750 Secretion of FGF21 and Follistatin Are Blocked by Pancreatic Clamp and Impaired in Type 2
751 Diabetes. *J. Clin. Endocrinol. Metab.* (May 2016). doi: 10.1210/jc.2016-1681.
- 752 61. **Hansen JS, Rutti S, Arous C, Clemmesen JO, Secher NH, Drescher A, Gonelle-Gispert C,**
753 **Halban PA, Pedersen BK, Weigert C, Bouzakri K, Plomgaard P.** Circulating Follistatin Is Liver-
754 Derived and Regulated by the Glucagon-to-Insulin Ratio. *J Clin Endocrinol Metab* 101: 550–
755 560, 2016.
- 756 62. **Hashimoto O, Funaba M, Sekiyama K, Doi S, Shindo D, Satoh R, Itoi H, Oiwa H, Morita M,**
757 **Suzuki C, Sugiyama M, Yamakawa N, Takada H, Matsumura S, Inoue K, Oyadomari S, Sugino**
758 **H, Kurisaki A.** Activin E Controls Energy Homeostasis in Both Brown and White Adipose
759 Tissues as a Hepatokine. *Cell Rep* 25: 1193–1203, 2018.
- 760 63. **Hayashino Y, Jackson JL, Hirata T, Fukumori N, Nakamura F, Fukuhara S, Tsujii S, Ishii H.**
761 Effects of exercise on C-reactive protein, inflammatory cytokine and adipokine in patients
762 with type 2 diabetes: A meta-analysis of randomized controlled trials. *Metabolism* 63: 431–
763 440, 2014.
- 764 64. **Hoene M, Franken H, Fritsche L, Lehmann R, Pohl AK, Häring HU, Zell A, Schleicher ED,**
765 **Weigert C.** Activation of the mitogen-activated protein kinase (MAPK) signalling pathway in
766 the liver of mice is related to plasma glucose levels after acute exercise. *Diabetologia* 53:
767 1131–1141, 2010.
- 768 65. **Hoene M, Lehmann R, Hennige AM, Pohl AK, Häring HU, Schleicher ED, Weigert C.** Acute
769 regulation of metabolic genes and insulin receptor substrates in the liver of mice by one single
770 bout of treadmill exercise. *J Physiol* 5871: 241–252, 2009.
- 771 66. **Hofmann M, Schober-Halper B, Oesen S, Franzke B, Tschann H, Bachl N, Strasser E-M, Quittan**
772 **M, Wagner K-H, Wessner B.** Effects of elastic band resistance training and nutritional
773 supplementation on muscle quality and circulating muscle growth and degradation factors of
774 institutionalized elderly women: the Vienna Active Ageing Study (VAAS). *Eur J Appl Physiol*
775 116: 885–897, 2016.

- 776 67. **Hwang H-J, Jung TW, Kim B-H, Hong HC, Seo JA, Kim SG, Kim NH, Choi KM, Choi DS, Baik SH,**
777 **Yoo HJ.** A dipeptidyl peptidase-IV inhibitor improves hepatic steatosis and insulin resistance
778 by AMPK-dependent and JNK-dependent inhibition of LECT2 expression. *Biochem Pharmacol*
779 98: 157–166, 2015.
- 780 68. **Ingerslev B, Hansen JS, Hoffmann C, Clemmesen JO, Secher NH, Scheler M, Hrabě de Angelis**
781 **M, Häring HU, Pedersen BK, Weigert C, Plomgaard P.** Angiopoietin-like protein 4 is an
782 exercise-induced hepatokine in humans, regulated by glucagon and cAMP. *Mol Metab* 6:
783 1286–1295, 2017.
- 784 69. **Ishikura K, Misu H, Kumazaki M, Takayama H, Matsuzawa-Nagata N, Tajima N, Chikamoto K,**
785 **Lan F, Ando H, Ota T, Sakurai M, Takeshita Y, Kato K, Fujimura A, Miyamoto K, Saito Y,**
786 **Kameo S, Okamoto Y, Takuwa Y, Takahashi K, Kidoya H, Takakura N, Kaneko S, Takamura T.**
787 Selenoprotein P as a diabetes-associated hepatokine that impairs angiogenesis by inducing
788 VEGF resistance in vascular endothelial cells. *Diabetologia* 57: 1968–1976, 2014.
- 789 70. **Ix JH, Biggs ML, Mukamal KJ, Kizer JR, Zieman SJ, Siscovick DS, Mozaffarian D, Jensen MK,**
790 **Nelson L, Ruderman N, Djousse L.** Association of fetuin-a with incident diabetes mellitus in
791 community-living older adults: the cardiovascular health study. *Circulation* 125: 2316–22,
792 2012.
- 793 71. **Izumiya Y, Bina HA, Ouchi N, Akasaki Y, Kharitononkov A, Walsh K.** FGF21 is an Akt-regulated
794 myokine. *FEBS Lett* 582: 3805–3810, 2008.
- 795 72. **Izumiya Y, Hopkins T, Morris C, Sato K, Zeng L, Viereck J, Hamilton JA, Ouchi N, LeBrasseur**
796 **NK, Walsh K.** Fast/Glycolytic Muscle Fiber Growth Reduces Fat Mass and Improves Metabolic
797 Parameters in Obese Mice. *Cell Metab* 7: 159–172, 2008.
- 798 73. **Janssen AWF, Katiraei S, Bartosinska B, Eberhard D, Willems van Dijk K, Kersten S.** Loss of
799 angiopoietin-like 4 (ANGPTL4) in mice with diet-induced obesity uncouples visceral obesity
800 from glucose intolerance partly via the gut microbiota. *Diabetologia* 61: 1–12, 2018.
- 801 74. **Kantartzis K, MacHann J, Schick F, Fritsche A, Häring HU, Stefan N.** The impact of liver fat vs
802 visceral fat in determining categories of prediabetes. *Diabetologia* 53: 882–889, 2010.
- 803 75. **Kawanishi N, Yano H, Mizokami T, Takahashi M, Oyanagi E, Suzuki K.** Exercise training
804 attenuates hepatic inflammation, fibrosis and macrophage infiltration during diet induced-
805 obesity in mice. *Brain Behav Immun* 26: 931–941, 2012.
- 806 76. **Kawanishi N, Yano H, Yokogawa Y, Suzuki K.** Exercise training inhibits inflammation in
807 adipose tissue via both suppression of macrophage infiltration and acceleration of phenotypic
808 switching from M1 to M2 macrophages in high-fat-diet-induced obese mice. *Exerc Immunol*
809 *Rev* 16: 105–118, 2010.
- 810 77. **Keating SE, Johnson NA, Mielke GI, Coombes JS.** A systematic review and meta-analysis of
811 interval training versus moderate-intensity continuous training on body adiposity. *Obes Rev*
812 18: 943–964, 2017.
- 813 78. **Kersten S, Lichtenstein L, Steenbergen E, Mudde K, Hendriks HFJ, Hesselink MK, Schrauwen**
814 **P, Müller M.** Caloric restriction and exercise increase plasma ANGPTL4 levels in humans via
815 elevated free fatty acids. *Arterioscler Thromb Vasc Biol* 29: 969–74, 2009.
- 816 79. **Kharitononkov A, Adams AC.** Inventing new medicines: The FGF21 story. *Mol. Metab.* 3
817 Elsevier: 221–229, 2014.

- 818 80. **Kharitononkov A, Shiyanova TL, Koester A, Ford AM, Micanovic R, Galbreath EJ, Sandusky**
819 **GE, Hammond LJ, Moyers JS, Owens RA, Gromada J, Brozinick JT, Hawkins ED, Wroblewski**
820 **VJ, Li D-S, Mehrbod F, Jaskunas SR, Shanafelt AB.** FGF-21 as a novel metabolic regulator. *J*
821 *Clin Invest* 115: 1627–35, 2005.
- 822 81. **Kim KH, Kim SH, Min YK, Yang HM, Lee JB, Lee MS.** Acute Exercise Induces FGF21 Expression
823 in Mice and in Healthy Humans. *PLoS One* 8, 2013.
- 824 82. **Koliaki C, Szendroedi J, Kaul K, Jelenik T, Nowotny P, Jankowiak F, Herder C, Carstensen M,**
825 **Krausch M, Knoefel WT, Schlensak M, Roden M.** Adaptation of Hepatic Mitochondrial
826 Function in Humans with Non-Alcoholic Fatty Liver Is Lost in Steatohepatitis. *Cell Metab* 21:
827 739–746, 2015.
- 828 83. **van der Kolk BW, Goossens GH, Jocken JW, Kersten S, Blaak EE.** Angiopoietin-Like Protein 4
829 and Postprandial Skeletal Muscle Lipid Metabolism in Overweight and Obese Prediabetics. *J*
830 *Clin Endocrinol Metab* 101: 2332–2339, 2016.
- 831 84. **Kong Z, Sun S, Liu M, Shi Q.** Short-Term High-Intensity Interval Training on Body Composition
832 and Blood Glucose in Overweight and Obese Young Women. *J Diabetes Res* 2016: 1–9, 2016.
- 833 85. **Köster A, Chao YB, Mosior M, Ford A, Gonzalez-DeWhitt PA, Hale JE, Li D, Qiu Y, Fraser CC,**
834 **Yang DD, Heuer JG, Jaskunas SR, Eacho P.** Transgenic angiopoietin-like (angptl)4
835 overexpression and targeted disruption of angptl4 and angptl3: regulation of triglyceride
836 metabolism. *Endocrinology* 146: 4943–50, 2005.
- 837 86. **de Kretser DM, Hedger MP, Loveland KL, Phillips DJ.** Inhibins, activins and follistatin in
838 reproduction. *Hum Reprod Update* 8: 529–541, 2002.
- 839 87. **Kurosu H, Choi M, Ogawa Y, Dickson AS, Goetz R, Eliseenkova A V, Mohammadi M,**
840 **Rosenblatt KP, Kliewer SA, Kuro-o M.** Tissue-specific expression of betaKlotho and fibroblast
841 growth factor (FGF) receptor isoforms determines metabolic activity of FGF19 and FGF21. *J*
842 *Biol Chem* 282: 26687–95, 2007.
- 843 88. **Lafferty MJ, Bradford KC, Erie DA, Neher SB.** Angiopoietin-like protein 4 inhibition of
844 lipoprotein lipase: evidence for reversible complex formation. *J Biol Chem* 288: 28524–34,
845 2013.
- 846 89. **Lee JH, Kang YE, Chang JY, Park KC, Kim H-W, Kim JT, Kim HJ, Yi H-S, Shong M, Chung HK, Kim**
847 **KS.** An engineered FGF21 variant, LY2405319, can prevent non-alcoholic steatohepatitis by
848 enhancing hepatic mitochondrial function. *Am J Transl Res* 8: 4750–4763, 2016.
- 849 90. **Lee S, Norheim F, Gulseth HL, Langleite TM, Kolnes KJ, Tangen DS, Stadheim HK, Gilfillan GD,**
850 **Holen T, Birkeland KI, Jensen J, Drevon CA.** Interaction between plasma fetuin-A and free
851 fatty acids predicts changes in insulin sensitivity in response to long-term exercise. *Physiol Rep*
852 5: e13183, 2017.
- 853 91. **Li H, Fang Q, Gao F, Fan J, Zhou J, Wang X, Zhang H, Pan X, Bao Y, Xiang K, Xu A, Jia W.**
854 Fibroblast growth factor 21 levels are increased in nonalcoholic fatty liver disease patients
855 and are correlated with hepatic triglyceride. *J Hepatol* 53: 934–40, 2010.
- 856 92. **Li J-Y, Chen X-X, Lu X-H, Zhang C-B, Shi Q-P, Feng L.** Elevated RBP4 plasma levels were
857 associated with diabetic retinopathy in type 2 diabetes. doi: 10.1042/BSR20181100.
- 858 93. **Li Y, Wong K, Walsh K, Gao B, Zang M.** Retinoic acid receptor β stimulates hepatic induction
859 of fibroblast growth factor 21 to promote fatty acid oxidation and control whole-body energy

- homeostasis in mice. *J Biol Chem* 288: 10490–504, 2013.
94. **Lichtenstein L, Berbée JFP, van Dijk SJ, van Dijk KW, Bensadoun A, Kema IP, Voshol PJ, Müller M, Rensen PCN, Kersten S.** Angptl4 upregulates cholesterol synthesis in liver via inhibition of LPL- and HL-dependent hepatic cholesterol uptake. *Arterioscler Thromb Vasc Biol* 27: 2420–7, 2007.
95. **Lin GG, Scott JG.** Reduction in the Incidence of Type 2 Diabetes With Lifestyle Intervention or Metformin. *N Engl J Med* 100: 130–134, 2012.
96. **Lin X, Braymer HD, Bray GA, York DA.** Differential expression of insulin receptor tyrosine kinase inhibitor (fetuin) gene in a model of diet-induced obesity. *Life Sci* 63: 145–53, 1998.
97. **Loyd C, Magrisso IJ, Haas M, Balusu S, Krishna R, Itoh N, Sandoval DA, Perez-Tilve D, Obici S, Habegger KM.** Fibroblast growth factor 21 is required for beneficial effects of exercise during chronic high-fat feeding. *J Appl Physiol* 121: 687–98, 2016.
98. **Lundsgaard A-M, Fritzen AM, Sjøberg KA, Myrmel LS, Madsen L, Wojtaszewski JFP, Richter EA, Kiens B.** Circulating FGF21 in humans is potently induced by short term overfeeding of carbohydrates. *Mol Metab* 6: 22–29, 2017.
99. **Malin SK, Mulya A, Fealy CE, Haus JM, Pagadala MR, Scelsi AR, Huang H, Flask CA, McCullough AJ, Kirwan JP.** Fetuin-A is linked to improved glucose tolerance after short-term exercise training in non-alcoholic fatty liver disease. *J Appl Physiol* 115: 988–994, 2013.
100. **Malin SK, Del Rincon JP, Huang H, Kirwan JP.** Exercise-induced lowering of fetuin-A may increase hepatic insulin sensitivity. *Med Sci Sports Exerc* 46: 2085–2090, 2014.
101. **Markan KR, Naber MC, Ameka MK, Anderegg MD, Mangelsdorf DJ, Kliewer SA, Mohammadi M, Potthoff MJ.** Circulating FGF21 is liver derived and enhances glucose uptake during refeeding and overfeeding. *Diabetes* 63: 4057–63, 2014.
102. **Marshall JPS, Estevez E, Kammoun HL, King EJ, Bruce CR, Drew BG, Qian H, Iliades P, Gregorevic P, Febbraio MA, Henstridge DC.** Skeletal muscle-specific overexpression of heat shock protein 72 improves skeletal muscle insulin-stimulated glucose uptake but does not alter whole body metabolism. *Diabetes Obes Metab* 20: 1928–1936, 2018.
103. **Mashili FL, Austin RL, Deshmukh AS, Fritz T, Caidahl K, Bergdahl K, Zierath JR, Chibalin A V, Moller DE, Kharitonov A, Krook A.** Direct effects of FGF21 on glucose uptake in human skeletal muscle: implications for type 2 diabetes and obesity. *Diabetes Metab Res Rev* 27: 286–97, 2011.
104. **Mathews S, Ren G, He X, Bowers R, Araya-Ramirez F, Littlefield L, Grandjean P.** Plasma fetuin-A and phosphofetuin-A (Ser312) responses to a single or short-term repeated bout of exercise in obese and normal-weight individuals (1028.2). *FASEB J* 28: 1028.2, 2014.
105. **Mathews ST, Chellam N, Srinivas PR, Cintron VJ, Leon MA, Goustin AS, Grunberger G.** α 2-HSG, a specific inhibitor of insulin receptor autophosphorylation, interacts with the insulin receptor. *Mol Cell Endocrinol* 164: 87–98, 2000.
106. **Mathews ST, Rakhade S, Zhou X, Parker GC, Coscina D V., Grunberger G.** Fetuin-null mice are protected against obesity and insulin resistance associated with aging. *Biochem Biophys Res Commun* 350: 437–443, 2006.
107. **Mathews ST, Singh GP, Ranalletta M, Cintron VJ, Qiang X, Goustin AS, Jen KLC, Charron MJ,**

- 901 **Jahnen-Dechent W, Grunberger G.** Improved insulin sensitivity and resistance to weight gain
902 in mice null for the Ahsg gene. *Diabetes* 51: 2450–2458, 2002.
- 903 108. **Meex RC, Hoy AJ, Morris A, Lancaster GI, Bruce CR, Watt Correspondence MJ, Brown RD, Lo**
904 **JCY, Burke M, Goode RJA, Kingwell BA, Kraakman MJ, Febbraio MA, Greve JW, Rensen SS,**
905 **Molloy MP, Watt MJ.** Fetuin B Is a Secreted Hepatocyte Factor Linking Steatosis to Impaired
906 Glucose Metabolism Cell Metabolism Resource Fetuin B Is a Secreted Hepatocyte Factor
907 Linking Steatosis to Impaired Glucose Metabolism. *Cell Metab* 22: 1078–1089, 2015.
- 908 109. **Meex RCR, Watt MJ.** Hepatokines: linking nonalcoholic fatty liver disease and insulin
909 resistance. *Nat Rev Endocrinol* 13: 509–520, 2017.
- 910 110. **Misu H, Takamura T, Takayama H, Hayashi H, Matsuzawa-Nagata N, Kurita S, Ishikura K,**
911 **Ando H, Takeshita Y, Ota T, Sakurai M, Yamashita T, Mizukoshi E, Yamashita T, Honda M,**
912 **Miyamoto K, Kubota T, Kubota N, Kadowaki T, Kim H-J, Lee I, Minokoshi Y, Saito Y,**
913 **Takahashi K, Yamada Y, Takakura N, Kaneko S.** A Liver-Derived Secretory Protein,
914 Selenoprotein P, Causes Insulin Resistance. *Cell Metab* 12: 483–495, 2010.
- 915 111. **Misu H, Takayama H, Saito Y, Mita Y, Kikuchi A, Ishii K-A, Chikamoto K, Kanamori T, Tajima**
916 **N, Lan F, Takeshita Y, Honda M, Tanaka M, Kato S, Matsuyama N, Yoshioka Y, Iwayama K,**
917 **Tokuyama K, Akazawa N, Maeda S, Takekoshi K, Matsugo S, Noguchi N, Kaneko S, Takamura**
918 **T.** Deficiency of the hepatokine selenoprotein P increases responsiveness to exercise in mice
919 through upregulation of reactive oxygen species and AMP-activated protein kinase in muscle.
920 *Nat Med* 23: 508–516, 2017.
- 921 112. **Moraes-Vieira PM, Yore MM, Dwyer PM, Syed I, Aryal P, Kahn BB.** RBP4 Activates Antigen-
922 Presenting Cells, Leading to Adipose Tissue Inflammation and Systemic Insulin Resistance. *Cell*
923 *Metab* 19: 512–526, 2014.
- 924 113. **Morrison AE, Zaccardi F, Khunti K, Davies MJ.** Causality between non-alcoholic fatty liver
925 disease and risk of cardiovascular disease and type 2 diabetes: A meta-analysis with bias
926 analysis. *Liver Int.* (December 2018). doi: 10.1111/liv.13994.
- 927 114. **Morville T, Sahl RE, Trammell SA, Svenningsen JS, Gillum MP, Helge JW, Clemmensen C.**
928 Divergent effects of resistance and endurance exercise on plasma bile acids, FGF19, and
929 FGF21 in humans. *JCI Insight* 3, 2018.
- 930 115. **Nishimura T, Nakatake Y, Konishi M, Itoh N.** Identification of a novel FGF, FGF-21,
931 preferentially expressed in the liver. *Biochim Biophys Acta* 1492: 203–6, 2000.
- 932 116. **Norheim F, Hjorth M, Langleite TM, Lee S, Holen T, Bindesbøll C, Stadheim HK, Gulseth HL,**
933 **Birkeland KI, Kielland A, Jensen J, Dalen KT, Drevon CA.** Regulation of angiopoietin-like
934 protein 4 production during and after exercise. *Physiol Rep* 2, 2014.
- 935 117. **Oliveira CP, de Lima Sanches P, de Abreu-Silva EO, Marcadenti A.** Nutrition and Physical
936 Activity in Nonalcoholic Fatty Liver Disease. *J Diabetes Res* 2016: 4597246, 2016.
- 937 118. **Ostrowski K, Rohde T, Zacho M, Asp S, Pedersen BK.** Evidence that interleukin-6 is produced
938 in human skeletal muscle during prolonged running. *J Physiol* 508: 949–953, 1998.
- 939 119. **Ou H-Y, Yang Y-C, Wu H-T, Wu J-S, Lu F-H, Chang C-J.** Serum fetuin-A concentrations are
940 elevated in subjects with impaired glucose tolerance and newly diagnosed type 2 diabetes.
941 *Clin Endocrinol (Oxf)* 75: 450–5, 2011.
- 942 120. **Ou H-Y, Yang Y-C, Wu H-T, Wu J-S, Lu F-H, Chang C-J.** Increased Fetuin-A Concentrations in

- 943 Impaired Glucose Tolerance with or without Nonalcoholic Fatty Liver Disease, But Not
944 Impaired Fasting Glucose. *J Clin Endocrinol Metab* 97: 4717–4723, 2012.
- 945 121. **Pal D, Dasgupta S, Kundu R, Maitra S, Das G, Mukhopadhyay S, Ray S, Majumdar SS,**
946 **Bhattacharya S.** Fetuin-A acts as an endogenous ligand of TLR4 to promote lipid-induced
947 insulin resistance. *Nat Med* 18: 1279–85, 2012.
- 948 122. **Park SE, Park C-Y, Sweeney G.** Biomarkers of insulin sensitivity and insulin resistance: Past,
949 present and future. *Crit Rev Clin Lab Sci* 52: 180–190, 2015.
- 950 123. **Pedersen BK.** Muscle as a secretory organ. *Compr Physiol* 3: 1337–62, 2013.
- 951 124. **Pedersen BK, Febbraio MA.** Muscles, exercise and obesity: skeletal muscle as a secretory
952 organ. *Nat Rev Endocrinol* 8: 457–465, 2012.
- 953 125. **Peter A, Kovarova M, Staiger H, Machann J, Schick F, Königsrainer A, Königsrainer I,**
954 **Schleicher E, Fritsche A, Häring H-U, Stefan N.** The hepatokines fetuin-A and fetuin-B are
955 upregulated in the state of hepatic steatosis and may differently impact on glucose
956 homeostasis in humans. *Am J Physiol Metab* 314: E266–E273, 2018.
- 957 126. **Petryszak R, Keays M, Tang YA, Fonseca NA, Barrera E, Burdett T, Füllgrabe A, Fuentes AM-P,**
958 **Jupp S, Koskinen S, Mannion O, Huerta L, Megy K, Snow C, Williams E, Barzine M, Hastings E,**
959 **Weisser H, Wright J, Jaiswal P, Huber W, Choudhary J, Parkinson HE, Brazma A.** Expression
960 Atlas update—an integrated database of gene and protein expression in humans, animals and
961 plants. *Nucleic Acids Res* 44: D746–D752, 2016.
- 962 127. **Pietsch J, Batra A, Stroh T, Fedke I, Glauben R, Okur B, Zeitz M, Siegmund B.** Toll-like
963 receptor expression and response to specific stimulation in adipocytes and preadipocytes: on
964 the role of fat in inflammation. *Ann N Y Acad Sci* 1072: 407–9, 2006.
- 965 128. **Polyzos SA, Kountouras J, Anastasilakis AD, Triantafyllou GA, Mantzoros CS.** Activin A and
966 follistatin in patients with nonalcoholic fatty liver disease. *Metabolism* 65: 1550–1558, 2016.
- 967 129. **Porter JW, Rowles JL, Fletcher JA, Zidon TM, Winn NC, McCabe LT, Park Y-M, Perfield JW,**
968 **Thyfault JP, Rector RS, Padilla J, Vieira-Potter VJ.** Anti-inflammatory effects of exercise
969 training in adipose tissue do not require FGF21. *J Endocrinol* 235: 97–109, 2017.
- 970 130. **Rahimi N, Sharif MAS, Goharian AR, Pour AH.** The effects of aerobic exercises and 25(OH) D
971 supplementation on GLP1 and DPP4 level in type II diabetic patients. *Int J Prev Med* 8: 56,
972 2017.
- 973 131. **Rinella ME.** Nonalcoholic fatty liver disease a systematic review. *JAMA - J. Am. Med. Assoc.:*
974 2015.
- 975 132. **Robciuc MR, Naukkarinen J, Ortega-Alonso A, Tyynismaa H, Raivio T, Rissanen A, Kaprio J,**
976 **Ehnholm C, Jauhiainen M, Pietiläinen KH.** Serum angiopoietin-like 4 protein levels and
977 expression in adipose tissue are inversely correlated with obesity in monozygotic twins. *J Lipid*
978 *Res* 52: 1575–1582, 2011.
- 979 133. **Roberts CK, Croymans DM, Aziz N, Butch AW, Lee CC.** Resistance training increases SHBG in
980 overweight/obese, young men. *Metabolism* 62: 725–733, 2013.
- 981 134. **Rodino-Klapac LR, Haidet AM, Kota J, Handy C, Kaspar BK, Mendell JR.** Inhibition of
982 myostatin with emphasis on follistatin as a therapy for muscle disease. *Muscle Nerve* 39: 283–
983 296, 2009.

- 984 135. **Sakr HF, Al-Hashem FH, El-Naby WM, Alkhateeb MA, Zaki MS, Refaey HM, Morsy MD.**
985 Preventive roles of swimming exercise and pioglitazone treatment on hepatic dysfunction in a
986 rat model of metabolic syndrome. *Can J Physiol Pharmacol* 92: 162–170, 2014.
- 987 136. **Samuel VT, Liu Z-X, Wang A, Beddow SA, Geisler JG, Kahn M, Zhang X, Monia BP, Bhanot S,**
988 **Shulman GI.** Inhibition of protein kinase C ϵ prevents hepatic insulin resistance in nonalcoholic
989 fatty liver disease. *J Clin Invest* 117: 739–745, 2007.
- 990 137. **Sargeant JA, Aithal GP, Takamura T, Misu H, Takayama H, Douglas JA, Turner MC, Stensel DJ,**
991 **Nimmo MA, Webb DR, Yates T, King JA.** The influence of adiposity and acute exercise on
992 circulating hepatokines in normal weight and overweight/obese men. *Appl. Physiol. Nutr.*
993 *Metab.* (December 2017). doi: 10.1139/apnm-2017-0639.
- 994 138. **Shen X, Yang L, Yan S, Zheng H, Liang L, Cai X, Liao M.** Fetuin A promotes lipotoxicity in β cells
995 through the TLR4 signaling pathway and the role of pioglitazone in anti-lipotoxicity. *Mol Cell*
996 *Endocrinol* 412: 1–11, 2015.
- 997 139. **Shi H, Kokoeva M V., Inouye K, Tzameli I, Yin H, Flier JS.** TLR4 links innate immunity and fatty
998 acid-induced insulin resistance. *J Clin Invest* 116: 3015–3025, 2006.
- 999 140. **Shimasaki S, Koga M, Esch F, Cooksey K, Mercado M, Koba A, Ueno N, Ying SY, Ling N,**
1000 **Guillemin R.** Primary structure of the human follistatin precursor and its genomic
1001 organization. *Proc Natl Acad Sci U S A* 85: 4218–22, 1988.
- 1002 141. **Silva RN, Bueno PG, Avó LRS, Nonaka KO, Selistre-Araújo HS, Leal AMO.** Effect of physical
1003 training on liver expression of activin A and follistatin in a nonalcoholic fatty liver disease
1004 model in rats. *Brazilian J Med Biol Res = Rev Bras Pesqui medicas e Biol* 47: 746–52, 2014.
- 1005 142. **Simó R, Sáez-López C, Barbosa-Desongles A, Hernández C, Selva DM.** Novel insights in SHBG
1006 regulation and clinical implications. *Trends Endocrinol Metab* 26: 376–383, 2015.
- 1007 143. **Slusher AL, Whitehurst M, Zoeller RF, Mock JT, Maharaj M, Huang CJ.** Attenuated fibroblast
1008 growth factor 21 response to acute aerobic exercise in obese individuals. *Nutr Metab*
1009 *Cardiovasc Dis* 25: 839–845, 2015.
- 1010 144. **Song MJ, Kim KH, Yoon JM, Kim JB.** Activation of Toll-like receptor 4 is associated with insulin
1011 resistance in adipocytes. *Biochem Biophys Res Commun* 346: 739–745, 2006.
- 1012 145. **Srinivas PR, Wagner AS, Lekkala R V, Deutsch DD, Leon MA, Goustin AS, Grunberger G.**
1013 Serum alpha 2-HS-glycoprotein is an inhibitor of the human insulin receptor at the tyrosine
1014 kinase level. *Mol Endocrinol* 7: 1445–1455, 1993.
- 1015 146. **Staiger H, Haas C, Machann J, Werner R, Weisser M, Schick F, Machicao F, Stefan N, Fritsche**
1016 **A, Haring H-U.** Muscle-Derived Angiopoietin-Like Protein 4 Is Induced by Fatty Acids via
1017 Peroxisome Proliferator-Activated Receptor (PPAR)- and Is of Metabolic Relevance in
1018 Humans. *Diabetes* 58: 579–589, 2009.
- 1019 147. **Staiger H, Keuper M, Berti L, Hrabe de Angelis M, Häring H-U.** Fibroblast Growth Factor 21-
1020 Metabolic Role in Mice and Men. *Endocr Rev* 38: 468–488, 2017.
- 1021 148. **Steensberg A, van Hall G, Osada T, Sacchetti M, Saltin B, Klarlund Pedersen B.** Production of
1022 interleukin-6 in contracting human skeletal muscles can account for the exercise-induced
1023 increase in plasma interleukin-6. *J Physiol* 529 Pt 1: 237–42, 2000.
- 1024 149. **Stefan N, Häring H-U.** The role of hepatokines in metabolism. *Nat Rev Endocrinol* 9: 144–152,

- 1025 2013.
- 1026 150. **Stefan N, Häring H-U.** Circulating fetuin-A and free fatty acids interact to predict insulin
1027 resistance in humans. *Nat Med* 19: 394–395, 2013.
- 1028 151. **Stefan N, Häring H-U, Cusi K.** Non-alcoholic fatty liver disease: causes, diagnosis,
1029 cardiometabolic consequences, and treatment strategies. *Lancet Diabetes Endocrinol* 0, 2018.
- 1030 152. **Stefan N, Hennige AM, Staiger H, Machann J, Schick F, Kröber SM, Machicao F, Fritsche A,**
1031 **Häring H-U.** Alpha2-Heremans-Schmid glycoprotein/fetuin-A is associated with insulin
1032 resistance and fat accumulation in the liver in humans. *Diabetes Care* 29: 853–7, 2006.
- 1033 153. **Stefan N, Kantartzis K, Machann J, Schick F, Thamer C, Rittig K, Balletshofer B, Machicao F,**
1034 **Fritsche A, Häring HU.** Identification and characterization of metabolically benign obesity in
1035 humans. *Arch Intern Med* 168: 1609–1616, 2008.
- 1036 154. **Takahashi H, Kotani K, Tanaka K, Eguchi Y, Anzai K.** Therapeutic Approaches to Nonalcoholic
1037 Fatty Liver Disease: Exercise Intervention and Related Mechanisms. *Front Endocrinol*
1038 *(Lausanne)* 9: 588, 2018.
- 1039 155. **Takata H, Ikeda Y, Suehiro T, Ishibashi A, Inoue M, Kumon Y, Terada Y.** High glucose induces
1040 transactivation of the alpha2-HS glycoprotein gene through the ERK1/2 signaling pathway. *J*
1041 *Atheroscler Thromb* 16: 448–56, 2009.
- 1042 156. **Tan BK, Hallschmid M, Adya R, Kern W, Lehnert H, Randeve HS.** Fibroblast growth factor 21
1043 (FGF21) in human cerebrospinal fluid: Relationship with plasma FGF21 and body adiposity.
1044 *Diabetes* 60: 2758–2762, 2011.
- 1045 157. **Taniguchi H, Tanisawa K, Sun X, Cao Z-B, Oshima S, Ise R, Sakamoto S, Higuchi M.**
1046 Cardiorespiratory fitness and visceral fat are key determinants of serum fibroblast growth
1047 factor 21 concentration in Japanese men. *J Clin Endocrinol Metab* 99: E1877–84, 2014.
- 1048 158. **Taniguchi H, Tanisawa K, Sun X, Kubo T, Higuchi M.** Endurance Exercise Reduces Hepatic Fat
1049 Content and Serum Fibroblast Growth Factor 21 Levels in Elderly Men. *J Clin Endocrinol Metab*
1050 101: 191–8, 2016.
- 1051 159. **Tanimura Y, Aoi W, Takanami Y, Kawai Y, Mizushima K, Naito Y, Yoshikawa T.** Acute exercise
1052 increases fibroblast growth factor 21 in metabolic organs and circulation. *Physiol Rep* 4: 426–
1053 437, 2016.
- 1054 160. **Tao R, Wang C, Stöhr O, Qiu W, Hu Y, Miao J, Dong XC, Leng S, Stefater M, Stylopoulos N, Lin**
1055 **L, Copps KD, White MF.** Inactivating hepatic follistatin alleviates hyperglycemia. *Nat. Med.* (
1056 June 2018). doi: 10.1038/s41591-018-0048-0.
- 1057 161. **Thoma C, Day CP, Trenell MI.** Lifestyle interventions for the treatment of non-alcoholic fatty
1058 liver disease in adults: A systematic review. *J. Hepatol.* 56 Elsevier: 255–266, 2012.
- 1059 162. **Trepanowski JF, Mey J, Varady KA.** Fetuin-A: a novel link between obesity and related
1060 complications. *Int J Obes* 39: 734–741, 2015.
- 1061 163. **Ungerleider NA, Bonomi LM, Brown ML, Schneyer AL.** Increased activin bioavailability
1062 enhances hepatic insulin sensitivity while inducing hepatic steatosis in male mice.
1063 *Endocrinology* 154: 2025–33, 2013.
- 1064 164. **Wang Y, Liu LM, Wei L, Ye WW, Meng XY, Chen F, Xiao Q, Chen JY, Zhou Y.** Angiopoietin-like

1065 protein 4 improves glucose tolerance and insulin resistance but induces liver steatosis in high-
1066 fat-diet mice. *Mol Med Rep* 14: 3293–3300, 2016.

1067 165. **Whitham M, Febbraio MA.** The ever-expanding myokine: discovery challenges and
1068 therapeutic implications. *Nat Rev Drug Discov* 15: 719–729, 2016.

1069 166. **Widhalm K, Ghods E.** Nonalcoholic fatty liver disease: a challenge for pediatricians. *Int J Obes*
1070 *(Lond)* 34: 1451–1467, 2010.

1071 167. **Wu H-T, Lu F-H, Ou H-Y, Su Y-C, Hung H-C, Wu J-S, Yang Y-C, Wu C-L, Chang C-J.** The role of
1072 Hepassocin in the development of non-alcoholic fatty liver disease. *J Hepatol* 59: 1065–1072,
1073 2013.

1074 168. **Wu H-T, Ou H-Y, Hung H-C, Su Y-C, Lu F-H, Wu J-S, Yang Y-C, Wu C-L, Chang C-J.** A novel
1075 hepatokine, HFREP1, plays a crucial role in the development of insulin resistance and type 2
1076 diabetes. *Diabetologia* 59: 1732–42, 2016.

1077 169. **Xia M, Liu Y, Guo H, Wang D, Wang Y, Ling W.** Retinol binding protein 4 stimulates hepatic
1078 sterol regulatory element-binding protein 1 and increases lipogenesis through the peroxisome
1079 proliferator-activated receptor- γ coactivator 1 β -dependent pathway. *Hepatology* 58: 564–
1080 575, 2013.

1081 170. **Xiong X, Wang Q, Wang S, Zhang J, Liu T, Guo L, Yu Y, Lin JD.** Mapping the molecular
1082 signatures of diet-induced NASH and its regulation by the hepatokine Tsukushi. *Mol Metab*
1083 20: 128–137, 2018.

1084 171. **Xu A, Lam MC, Chan KW, Wang Y, Zhang J, Hoo RLC, Xu JY, Chen B, Chow W-S, Tso AWK, Lam**
1085 **KSL.** Angiopoietin-like protein 4 decreases blood glucose and improves glucose tolerance but
1086 induces hyperlipidemia and hepatic steatosis in mice. *Proc Natl Acad Sci* 102: 6086–6091,
1087 2005.

1088 172. **Xu J, Lloyd DJ, Hale C, Stanislaus S, Chen M, Sivits G, Vonderfecht S, Hecht R, Li Y-S, Lindberg**
1089 **RA, Chen J-L, Young Jung D, Zhang Z, Ko H-J, Kim JK, Veniant MM.** Fibroblast Growth Factor
1090 21 Reverses Hepatic Steatosis, Increases Energy Expenditure, and Improves Insulin Sensitivity
1091 in Diet-Induced Obese Mice. *Diabetes* 58: 250–259, 2009.

1092 173. **Yan H, Xia M, Chang X, Xu Q, Bian H, Zeng M, Rao S, Yao X, Tu Y, Jia W, Gao X.** Circulating
1093 fibroblast growth factor 21 levels are closely associated with hepatic fat content: A cross-
1094 sectional study. *PLoS One* 6: e24895, 2011.

1095 174. **Yang SJ, Hong HC, Choi HY, Yoo HJ, Cho GJ, Hwang TG, Baik SH, Choi DS, Kim SM, Choi KM.**
1096 Effects of a three-month combined exercise programme on fibroblast growth factor 21 and
1097 fetuin-A levels and arterial stiffness in obese women. *Clin Endocrinol (Oxf)* 75: 464–469, 2011.

1098 175. **Yilmaz Y, Yonal O, Kurt R, Ari F, Oral AY, Celikel CA, Korkmaz S, Ulukaya E, Ozdogan O,**
1099 **Imeryuz N, Avsar E, Kalayci C.** Serum fetuin A/alpha2HS-glycoprotein levels in patients with
1100 non-alcoholic fatty liver disease: relation with liver fibrosis. *Ann Clin Biochem* 47: 549–553,
1101 2010.

1102 176. **Yndestad A, Haukeland JW, Dahl TB, Bjørø K, Gladhaug IP, Berge C, Damås JK, Haaland T,**
1103 **Løberg EM, Linnestad P, Birkeland K, Konopski Z, Halvorsen B, Berge RK, Aukrust P.** A
1104 Complex Role of Activin A in Non-Alcoholic Fatty Liver Disease. *Am J Gastroenterol* 104: 2196–
1105 2205, 2009.

1106 177. **Yu H, Xia F, Lam KSL, Wang Y, Bao Y, Zhang J, Gu Y, Zhou P, Lu J, Jia W, Xu A.** Circadian

1107 Rhythm of Circulating Fibroblast Growth Factor 21 Is Related to Diurnal Changes in Fatty Acids
1108 in Humans. *Clin Chem* 57: 691–700, 2011.

1109 178. **Zarei M, Barroso E, Palomer X, Dai J, Rada P, Quesada-López T, Escolà-Gil JC, Cedó L, Zali MR,**
1110 **Molaei M, Dabiri R, Vázquez S, Pujol E, Valverde ÁM, Villarroya F, Liu Y, Wahli W, Vázquez-**
1111 **Carrera M.** Hepatic regulation of VLDL receptor by PPAR β/δ and FGF21 modulates non-
1112 alcoholic fatty liver disease. *Mol Metab* 8: 117–131, 2018.

1113 179. **Zhang X, Yeung DCY, Karpisek M, Stejskal D, Zhou Z-G, Liu F, Wong RLC, Chow W-S, Tso**
1114 **AWK, Lam KSL, Xu A.** Serum FGF21 levels are increased in obesity and are independently
1115 associated with the metabolic syndrome in humans. *Diabetes* 57: 1246–53, 2008.

1116 180. **Zhao C, Qiao C, Tang R-H, Jiang J, Li J, Martin CB, Bulaklak K, Li J, Wang DW, Xiao X.**
1117 Overcoming Insulin Insufficiency by Forced Follistatin Expression in β -cells of db/db Mice. *Mol*
1118 *Ther* 23: 866–874, 2015.

1119 181. **Zhao J, Xiong X, Li Y, Liu X, Wang T, Zhang H, Jiao Y, Jiang J, Zhang H, Tang Q, Gao X, Li X, Lu**
1120 **Y, Liu B, Hu C, Li X.** Hepatic F-Box Protein FBXW7 Maintains Glucose Homeostasis Through
1121 Degradation of Fetuin-A. *Diabetes* 67: 818–830, 2018.

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Table 1. Metabolic roles of hepatokines and their systemic regulation by metabolic diseases and exercise.

Hepatokines	Metabolic roles	Biomarkers in metabolic diseases	Acute exercise		Chronic exercise (metabolic diseases)	Refs
			Healthy	Metabolic diseases		
α 1-microglobulin	Promotes adipose tissue inflammation	-	-	-	-	(56)
Activin β E	Stimulates energy expenditure and increases insulin sensitivity through brown and beige adipocyte activation	-	-	-	-	(62)
ANGPTL4	Major role in lipid metabolism, systemic levels of lipids and liver steatosis.	?	↗	↗	↗	(68, 116, 164, 171)
DPP4	Promotes adipose tissue inflammation and insulin resistance	↗ (if inflammation)	-	-	↘	(50, 130)
EDA	Promotes insulin resistance	↗ (mouse only)	-	-	-	(10)
Fam3C	Improves insulin resistance and fatty liver, suppressing hepatic gluconeogenesis	-	-	-	-	(26)
Fetuin A	Promotes adipose tissue inflammation, lipotoxicity of β -cells and insulin resistance	↗	-	↔		(137, 162)
Fetuin B	Impairs glucose tolerance	↗	-	-	-	(108, 125)
FGF21	Improves glucose tolerance, insulin sensitivity, steatosis, lipids profile and β -cell function. Promote adipose tissue browning	↗	↗	↔	?	(59, 60, 143, 147)
Follistatin	Promotes glucose metabolism disruption by enhancing hepatic glucose production	↗	↗	↔	-	(57, 58, 60, 160)
Hepassocin/HFREP1	Promotes insulin resistance and hepatic lipid accumulation	↗	-	-	-	(167, 168)
HSP72	Promotes insulin sensitivity and mitochondrial function, reduces hepatic lipid accumulation and inflammation	↘	↗	-	-	(6, 28, 40, 102)

LECT2	Promotes insulin resistance, hepatic lipid accumulation and inflammation	↗	↔	↔		(67, 137)
MSP	Promotes hepatic inflammation but conversely inhibit hepatic lipid accumulation and regulates hepatic gluconeogenesis	-	-	-	-	(23)
RBP 4	Promotes hepatic lipid accumulation and adipose tissue inflammation	↗	↔	↗	?	(92, 112, 169)
Selenoprotein P	Impair insulin signalling and secretion, and dysregulate glucose metabolism. <i>Deficiency of selenoprotein P increases exercise responsiveness through upregulation of reactive oxygen species in muscle.</i>	↗	↔	↔	↔	(111, 137)
SHBG	Prevent obesity and fatty liver suppresses inflammation and lipid accumulation in adipose tissue	↘	-	-	↗	(133, 142)
Tsukushi	Decrease adipose tissue thermogenesis	↗ (mouse only)	-	-	-	(170)

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1126 ↗ Systemic increase; ↘ Systemic decrease; ↔ No change; - No data; ? Conflicting results; ANGPTL4, angiopoietin-like protein 4; DPP4,

1127 Dipeptidyl peptidase 4; EDA, ectodysplasin A; Fam3C, Family with sequence similarity 3C; FGF21, Fibroblast growth factor 21; HFREP1,

1128 Hepatocyte-derived fibrinogen-related protein 1; HSP72, Heat shock protein 72; LECT2, Leukocyte cell-derived chemotaxin-2; MSP,

1129 Macrophage stimulating protein; RBP4, Retinol-binding protein 4; SHBG, Sex Hormone Binding Globulin.

1130 **Figure legend**

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1132 **Fig. 1 Exercise-induced hepatokines production and release.** Prolonged endurance
1133 exercise promotes an increase in glucagon-to-insulin ratio and FFA. This results in the
1134 activation of two distinct pathways ATF4/cAMP/PPAR α and cAMP that trigger FGF21, Fst
1135 and ANGPTL4 production and release. This secretion is altered by metabolic diseases and
1136 nutritional state (fasting *vs* fed). Other mechanisms might participate in the production of
1137 exercise-induced hepatokine such as FGF21, Fst, ANGPTL4 and Fetuin-A.

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Tsukushi	Decrease adipose tissue thermogenesis	↗ (mouse only)	-	-	-	(170)

↗ Systemic increase; ↘ Systemic decrease; ↔ No change; - No data; ? Conflicting results; ANGPTL4, angiopoietin-like protein 4; DPP4, Dipeptidyl peptidase 4; EDA, ectodysplasin A; Fam3C, Family with sequence similarity 3C; FGF21, Fibroblast growth factor 21; HFREP1, Hepatocyte-derived fibrinogen-related protein 1; HSP72, Heat shock protein 72; LECT2, Leukocyte cell-derived chemotaxin-2; MSP, Macrophage stimulating protein; RBP4, Retinol-binding protein 4; SHBG, Sex Hormone Binding Globulin.